

09/586747

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NEWS	3	Jan 29	FSTA has been reloaded and moves to weekly updates
NEWS	4	Feb 01	DKILIT now produced by FIZ Karlsruhe and has a new update frequency
NEWS	5	Feb 19	Access via Tymnet and SprintNet Eliminated Effective 3/31/02
NEWS	6	Mar 08	Gene Names now available in BIOSIS
NEWS	7	Mar 22	TOXLIT no longer available
NEWS	8	Mar 22	TRCTHERMO no longer available
NEWS	9	Mar 28	US Provisional Priorities searched with P in CA/CAPLUS and USPATFULL
NEWS	10	Mar 28	LIPINSKI/CALC added for property searching in REGISTRY
NEWS	11	Apr 02	PAPERCHEM no longer available on STN. Use PAPERCHEM2 instead.
NEWS	12	Apr 08	"Ask CAS" for self-help around the clock
NEWS	13	Apr 09	BEILSTEIN: Reload and Implementation of a New Subject Area
NEWS	14	Apr 09	ZDB will be removed from STN
NEWS	15	Apr 19	US Patent Applications available in IFICDB, IFIPAT, and IFIUDB
NEWS	16	Apr 22	Records from IP.com available in CAPLUS, HCAPLUS, and ZCAPLUS
NEWS	17	Apr 22	BIOSIS Gene Names now available in TOXCENTER
NEWS	18	Apr 22	Federal Research in Progress (FEDRIP) now available
NEWS	19	Jun 03	New e-mail delivery for search results now available
NEWS	20	Jun 10	MEDLINE Reload
NEWS	21	Jun 10	PCTFULL has been reloaded
NEWS	22	Jul 02	FOREGE no longer contains STANDARDS file segment
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=> file medicine

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FILE 'USPAT2' ENTERED AT 09:57:49 ON 16 JUL 2002
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=> s PLGA AND microsphere? and hiv

14 FILES SEARCHED...

31 FILES SEARCHED...

L1 157 PLGA AND MICROSPHERE? AND HIV

09/586747

=> S L1 AND PD<1996
4 FILES SEARCHED...
'1996' NOT A VALID FIELD CODE
9 FILES SEARCHED...
'1996' NOT A VALID FIELD CODE
'1996' NOT A VALID FIELD CODE
16 FILES SEARCHED...
20 FILES SEARCHED...
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26 FILES SEARCHED...
'1996' NOT A VALID FIELD CODE
'1996' NOT A VALID FIELD CODE
31 FILES SEARCHED...
L2 20 L1 AND PD<1996

=> D L2 1-20

L2 ANSWER 1 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AN 1994:504304 BIOSIS
DN PREV199497517304
TI Characterization of V3 BRU peptide-loaded small **PLGA**
microspheres prepared by a (w-1/o)w-2 emulsion solvent evaporation
method.
AU Prieto, Maria Jose Blanco; Delie, Florence; Fattal, Elias; Tartar, Andre;
Puisieux, Francis; Gulik, Annette; Couvreur, Patrick (1)
CS (1) Lab. Physico-Chimie-Pharmacotechnie-Biopharmacie, URA CNRS 1218, Fac.
Pharmacie, 5 Rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex
France
SO International Journal of Pharmaceutics (Amsterdam), (1994) Vol. 111, No.
2, pp. 137-145.
ISSN: 0378-5173.
DT Article
LA English

L2 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1995:652539 CAPLUS
DN 123:40954
TI Microencapsulation of antigens in lactide/glycolide copolymer (
PLGA) for use as vaccines
IN Cleland, Jeffrey L.; Lim, Amy; Powell, Michael Frank
PA Genentech, Inc., USA
SO PCT Int. Appl., 57 pp.
CODEN: PIXXD2

DT Patent
LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	-----		-----	-----	-----
PI	WO 9511010	A1	19950427	WO 1994-US11753	19941013 <--
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2172509	AA	19950427	CA 1994-2172509	19941013 <--
	AU 9479807	A1	19950508	AU 1994-79807	19941013 <--
	EP 724432	A1	19960807	EP 1994-930794	19941013
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 09504027	T2	19970422	JP 1994-512118	19941013

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PRAI US 1993-141796 19931022
US 1993-143555 19931025
WO 1994-US11753 19941013

L2 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1995:260433 CAPLUS
DN 122:38660
TI Development of a single-shot subunit vaccine for **HIV-1**
AU Cleland, Jeffrey L.; Powell, Michael F.; Lim, Amy; Barron, Lorena; Berman, Phillip W.; Eastman, Donna J.; Nunberg, Jack H.; Wrin, Terri; Vennari, Joann C.
CS Department of Pharmaceutical Research and Development, Genentech, Inc., San Francisco, CA, 94080, USA
SO AIDS Res. Hum. Retroviruses (1994), 10(Suppl. 2), S21-S26
CODEN: ARHRE7; ISSN: 0889-2229
DT Journal
LA English

L2 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS
AN 1994:708124 CAPLUS
DN 121:308124
TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w1/o)w2 emulsion solvent evaporation method
AU Prieto, Maria Jose Blanco; Delie, Florence; Fattal, Elias; Tartar, Andre; Puisieux, Francis; Gulik, Annette; Couvreur, Patrick
CS Laboratoire Physico-Chimie-Pharmacotechnie-Biopharmacie, URA CNRS 1218, Faculte de Pharmacie, 5, Rue Jean Baptiste Clement, Chatenay Malabry, 92296, Fr.
SO Int. J. Pharm. (1994), 111(2), 137-45
CODEN: IJPHDE; ISSN: 0378-5173
DT Journal
LA English

L2 ANSWER 5 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 94369172 EMBASE
DN 1994369172
TI Development of a single-shot subunit vaccine for **HIV-1**.
AU Cleland J.L.; Powell M.F.; Lim A.; Barron L.; Berman P.W.; Eastman D.J.; Nunberg J.H.; Wrin T.; Vennari J.C.
CS Dept. of Pharmaceutical Res./Devt., Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080, United States
SO AIDS Research and Human Retroviruses, (1994) 10/SUPPL. 2 (S21-S26).
ISSN: 0889-2229 CODEN: ARHRE7
CY United States
DT Journal; Conference Article
FS 004 Microbiology
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English
SL English

L2 ANSWER 6 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
AN 94285036 EMBASE
DN 1994285036
TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w1/o)w2 emulsion solvent evaporation method.
AU Blanco Prieto M.J.; Delie F.; Fattal E.; Tartar A.; Puisieux F.; Gulik A.;

09/586747

Couvreur P.
CS Lab.Phys.Chim.PharmacoTech./Biophar., URA CNRS 1218, Faculte de Pharmacie,
5, Rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex, France
SO International Journal of Pharmaceutics, (1994) 111/2 (137-145).
ISSN: 0378-5173 CODEN: IJPHDE
CY Netherlands
DT Journal; Article
FS 037 Drug Literature Index
LA English
SL English

L2 ANSWER 7 OF 20 IPA COPYRIGHT 2002 ASHP

AN 94:14201 IPA
DN 33-02724
TI Characterization of V3-BRU peptide-loaded small **PLGA**
microspheres prepared by a (w1/o)w2 emulsion solvent evaporation
method
AU Blanco Prieto, M. J.; Delie, F.; Fattal, E.; Tartar, A.; Couvreur, P.; et
al
CS Lab. Physico-Chimie-Pharmacotechnie-Biopharm., URA CNRS 1218, Fac. de
Pharm., 5 rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex, France
SO International Journal of Pharmaceutics (Netherlands), (Oct 20 1994
) Vol. 111, pp. 137-145. 12 Refs.
CODEN: IJPHDE; ISSN: 0378-5173.
DT Journal
LA English

L2 ANSWER 8 OF 20 LIFESCI COPYRIGHT 2002 CSA
AN 95:48349 LIFESCI
TI Development of a single-shot subunit vaccine for **HIV-1**
AU Cleland, J.L.; Powell, M.F.; Lim, A.; Barron, L.; Berman, P.W.; Eastman,
D.J.; Nunberg, J.H.; Wrin, T.; Vennari, J.C.
CS Dep. Pharm. Res. and Dev. Genentech, Inc., 460 Pt. San Bruno Blvd., South
San Francisco, CA 94080, USA
SO AIDS RES. HUM. RETROVIRUSES, (1994) vol. 10, no. 2 suppl., pp.
S21-S26.
ISSN: 0889-2229.
DT Journal
FS V
LA English
SL English

L2 ANSWER 9 OF 20 COPYRIGHT 2002 Gale Group

AN 94:416976 NLDB
TI Booster' Compound Helps Effectiveness
SO High Tech Separations News, (Dec 1994) Vol. 7, No. 7.
ISSN: 1046-039X.
PB Business Communications Company, Inc
DT Newsletter
LA English
WC 429

L2 ANSWER 10 OF 20 COPYRIGHT 2002 Gale Group

AN 94:368410 NLDB
TI **HIV/Vaccine - Microsphere** Drug Delivery
SO Vaccine Weekly, (14 Nov 1994) .
ISSN: 1074-2921.

09/586747

PB Charles W Henderson
DT Newsletter
LA English
WC 309

L2 ANSWER 11 OF 20 COPYRIGHT 2002 Gale Group

AN 94:365631 NLDB
TI HIV/Vaccine - **Microsphere** Drug Delivery
SO AIDS Weekly, (14 Nov 1994) .
ISSN: 1069-1456.
PB CW Henderson, Publisher
DT Newsletter
LA English
WC 309

L2 ANSWER 12 OF 20 COPYRIGHT 2002 Gale Group

AN 94:315589 NLDB
TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for
HIV-1
SO Vaccine Weekly, (12 Sep 1994) .
ISSN: 1074-2921.
PB Charles W Henderson
DT Newsletter
LA English
WC 326

L2 ANSWER 13 OF 20 COPYRIGHT 2002 Gale Group

AN 94:314412 NLDB
TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for
HIV-1
SO AIDS Weekly, (12 Sep 1994) .
PB CW Henderson, Publisher
DT Newsletter
LA English
WC 326

L2 ANSWER 14 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

AN 94:745601 SCISEARCH
GA The Genuine Article (R) Number: PT118
TI DEVELOPMENT OF A SINGLE-SHOT SUBUNIT VACCINE FOR **HIV-1**
AU CLELAND J L (Reprint); POWELL M F; LIM A; BARRON L; BERMAN P W; EASTMAN D
J; NUNBERG J H; WRIN T; VENNARI J C
CS GENENTECH INC, DEPT PHARMACEUT RES & DEV, 460 PT SAN BRUNO BLVD, S SAN
FRANCISCO, CA, 94080 (Reprint); GENENTECH INC, DEPT IMMUNOL, S SAN
FRANCISCO, CA, 94080
CYA USA
SO AIDS RESEARCH AND HUMAN RETROVIRUSES, (1994) Vol. 10, Supp. 2,
pp. S21-S26.
ISSN: 0889-2229.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 14
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L2 ANSWER 15 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

AN 94:622970 SCISEARCH

09/586747

GA The Genuine Article (R) Number: PH828
TI CHARACTERIZATION OF V3 BRU PEPTIDE-LOADED SMALL **PLGA**
MICROSPHERES PREPARED BY A (W(1)/O)W(2) EMULSION SOLVENT
EVAPORATION METHOD
AU PRIETO M J B; DELIE F; FATTAL E; TARTAR A; PUISIEUX F; GULIK A; COUVREUR P
(Reprint)
CS FAC PHARM CHATENAY MALABRY, PHYS CHIM PHARMACOTECH BIOPHARM LAB, CNRS, URA
1218, F-92296 CHATENAY MALABRY, FRANCE (Reprint); FAC PHARM CHATENAY
MALABRY, PHYS CHIM PHARMACOTECH BIOPHARM LAB, CNRS, URA 1218, F-92296
CHATENAY MALABRY, FRANCE; CHIM BIOMOLEC LAB, CNRS, URA 1309, F-59019
LILLE, FRANCE; CTR GENET MOLEC, UPR A2420, F-91198 GIF SUR YVETTE, FRANCE
CYA FRANCE
SO INTERNATIONAL JOURNAL OF PHARMACEUTICS, (20 OCT 1994) Vol. 111,
No. 2, pp. 137-145.
ISSN: 0378-5173.
DT Article; Journal
FS LIFE
LA ENGLISH
REC Reference Count: 12
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L2 ANSWER 16 OF 20 USPATFULL
AN 1999:170238 USPATFULL
TI Nanoparticles and microparticles of non-linear hydrophilic-hydrophobic
multiblock copolymers
IN Domb, Abraham J., Efrat, Israel
Gref, Ruxandra, Nancy, France
Minamitake, Yoshiharu, Gumma, Japan
Peracchia, Maria Teresa, Parma, Italy
Langer, Robert S., Newton, MA, United States
PA Massachusetts Institute of Technology, Cambridge, MA, United States
(U.S. corporation)
PI US 6007845 19991228
WO 9503356 19950202 <--
AI US 1996-582993 19960325 (8)
WO 1994-US8287 19940722
19960122 PCT 371 date
19960122 PCT 102(e) date
DT Utility
FS Granted
LN.CNT 1368
INCL INCLM: 424/501.000
INCLS: 424/489.000; 424/497.000; 424/498.000; 424/502.000; 424/451.000;
424/462.000; 424/078.080; 514/772.300; 514/784.000; 514/963.000;
514/402.210; 428/402.240
NCL NCLM: 424/501.000
NCLS: 424/078.080; 424/451.000; 424/462.000; 424/489.000; 424/497.000;
424/498.000; 424/502.000; 428/402.210; 428/402.240; 514/772.300;
514/784.000; 514/963.000
IC [6]
ICM: A61K009-50
ICS: A61K009-14; A61K009-16; A61K009-48
EXF 424/78.08; 424/462; 424/451; 424/502; 424/498; 424/489; 424/497;
424/1-11; 424/9.4; 424/9.411; 424/9.5; 424/452; 514/772.3; 514/784;
514/963; 514/402.21; 428/402.21; 428/402.24; 428/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 17 OF 20 USPATFULL
AN 1998:95252 USPATFULL
TI Lymphatic delivery composition

09/586747

IN Davis, Stanley S., Nottingham, United Kingdom
Illum, Lisbeth, Nottingham, United Kingdom
Christy, Nicola, Nottingham, United Kingdom
Moghimi, Moein, Nottingham, United Kingdom
PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)
PI US 5792475 19980811
WO 9402122 19940203 <--
AI US 1995-374671 19950414 (8)
WO 1993-GB1596 19930728
19950414 PCT 371 date
19950414 PCT 102(e) date
PRAI GB 1992-16082 19920728
DT Utility
FS Granted
LN.CNT 1139
INCL INCLM: 424/489.000
INCLS: 424/490.000
NCL NCLM: 424/489.000
NCLS: 424/490.000
IC [6]
ICM: A61K009-51
EXF 424/490; 424/489; 428/402; 428/403
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 18 OF 20 USPATFULL
AN 96:120585 USPATFULL
TI Excipient stabilization of polypeptides treated with organic solvents
IN Cleland, Jeffrey L., San Carlos, CA, United States
Jones, Andrew J. S., San Mateo, CA, United States
PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)
PI US 5589167 19961231
WO 9419020 19940901 <--
AI US 1994-256187 19940408 (8)
WO 1994-US1666 19940217
19940408 PCT 371 date
19940408 PCT 102(e) date
RLI Continuation-in-part of Ser. No. US 1993-21421, filed on 23 Feb 1993, now abandoned
DT Utility
FS Granted
LN.CNT 912
INCL INCLM: 424/085.700
INCLS: 514/021.000; 530/351.000; 530/399.000
NCL NCLM: 424/085.700
NCLS: 514/021.000; 530/351.000; 530/399.000
IC [6]
ICM: A61K038-21
ICS: A61K038-27
EXF 424/85.7; 514/21; 530/351; 530/399
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 19 OF 20 USPATFULL
AN 95:45359 USPATFULL
TI Vaccines against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres**
IN Reid, Robert H., Kensington, MD, United States
Boedeker, Edgar C., Chevy Chase, MD, United States
van Hamont, John E., Shape, Belgium

09/586747

Setterstrom, Jean A., Takoma Park, MD, United States
PA The United States of America as represented by the Secretary of the
Army, Washington, DC, United States (U.S. government)
PI US 5417986 19950523 <--
AI US 1992-867301 19920410 (7)
RLI Continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991,
now abandoned which is a continuation-in-part of Ser. No. US
1991-690485, filed on 24 Apr 1991, now abandoned which is a
continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990,
now abandoned which is a continuation-in-part of Ser. No. US
1990-493597, filed on 15 Mar 1990, now abandoned which is a
continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility
FS Granted
LN.CNT 2736
INCL INCLM: 424/499.000
INCLS: 424/426.000; 424/455.000; 424/486.000; 424/488.000; 424/489.000;
424/444.000; 424/433.000; 424/470.000; 424/491.000; 424/422.000
NCL NCLM: 424/499.000
NCLS: 424/422.000; 424/426.000; 424/433.000; 424/444.000; 424/455.000;
424/470.000; 424/486.000; 424/488.000; 424/489.000; 424/491.000
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ICM: A61K009-50
ICS: A61K009-66; A61K009-26
EXF 424/499; 424/422; 424/85; 424/417; 424/450; 424/458; 424/469; 424/88;
424/89; 424/92; 424/863; 424/965
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 20 OF 20 USPATFULL
AN 90:78226 USPATFULL
TI Controlled release of macromolecular polypeptides
IN Eppstein, Deborah A., Palo Alto, CA, United States
Schryver, Brian B., Redwood City, CA, United States
PA Syntex (U.S.A.) Inc., Palo Alto, CA, United States (U.S. corporation)
PI US 4962091 19901009 <--
AI US 1986-866625 19860523 (6)
DT Utility
FS Granted
LN.CNT 1235
INCL INCLM: 514/002.000
INCLS: 514/021.000; 514/964.000; 424/078.000; 424/089.000; 424/092.000;
424/085.100; 424/085.200; 424/085.600; 424/085.800; 424/085.400
NCL NCLM: 424/085.200
NCLS: 424/085.100; 424/085.400; 424/085.600; 424/130.100; 424/178.100;
424/184.100; 424/193.100; 424/499.000; 514/002.000; 514/021.000;
514/964.000
IC [5]
ICM: A61K031-12
ICS: A61K047-00
EXF 424/78; 424/89; 424/85; 424/46; 424/92; 424/DIG.7; 424/486; 514/773;
514/772; 514/774; 514/775-778; 514/782; 514/951; 514/3-20; 514/958;
514/213; 514/21; 514/12-19; 514/2; 514/964
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

=> D L2 1-20 AB, KWIC, BIB

L2 ANSWER 1 OF 20 BIOSIS COPYRIGHT 2002 BIOLOGICAL ABSTRACTS INC.
AB This paper describes the conditions of preparation of poly(lactide-
coglycolide) **microspheres** with a mean diameter lower than 10

mu-m obtained by a (w-1/o)w-2 emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of HIV, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis of the release kinetics was carried out in phosphate buffer (PBS), in artificial gastric and intestinal medium. V3 BRU release in PBS was slow, reaching a plateau at 24 h corresponding to 25% of drug release. In addition, V3 BRU was not released in gastric medium within 4 h whereas under the same time conditions, 60% of the drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for HIV vaccination.

- TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w-1/o)w-2 emulsion solvent evaporation method.
- SO International Journal of Pharmaceutics (Amsterdam), (1994) Vol. 111, No. 2, pp. 137-145.
ISSN: 0378-5173.
- AB This paper describes the conditions of preparation of poly(lactide-coglycolide) **microspheres** with a mean diameter lower than 10 mu-m obtained by a (w-1/o)w-2 emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of HIV, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis. . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for HIV vaccination.
- AN 1994:504304 BIOSIS
- DN PREV199497517304
- TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w-1/o)w-2 emulsion solvent evaporation method.
- AU Prieto, Maria Jose Blanco; Delie, Florence; Fattal, Elias; Tartar, Andre; Puisieux, Francis; Gulik, Annette; Couvreur, Patrick (1)
- CS (1) Lab. Physico-Chimie-Pharmacotechnie-Biopharmacie, URA CNRS 1218, Fac. Pharmacie, 5 Rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex France
- SO International Journal of Pharmaceutics (Amsterdam), (1994) Vol. 111, No. 2, pp. 137-145.
ISSN: 0378-5173.
- DT Article
- LA English
- L2 ANSWER 2 OF 20 CAPLUS COPYRIGHT 2002 ACS
- AB Antigens are encapsulated in **PLGA microspheres** for use as vaccines. The wt. ratio of lactide to glycolide is (100:1)-(1:100), the inherent viscosity of the polymer is 0.1-1.2 dL/g, and the median diam. of the **microspheres** is 20-100 nm. The antigen is released

in a triphasic pattern: 0.5-95% is released in an initial burst, 0-50% is released over a period of 1-180 days, and the remainder is released in a 2nd burst after 1-180 days. Such **microspheres** can also contain adjuvants, e.g. QS 21. Mixts. of **microspheres** are provided which release antigen at desired intervals to provide boosts with antigen. The time until 2nd burst could be manipulated by varying the monomer ratio in the polymer. Microencapsulation of recombinant **HIV** glycoprotein gp120 did not alter its conformation, as shown by its degree of aggregation and hydrophobicity.

TI Microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines

PI WO 9511010 A1 **19950427**

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9511010	A1	19950427	WO 1994-US11753	19941013

PI WO 9511010 A1 19950427

WO 1994-US11753 19941013 <--

W: AU, CA, JP

RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

CA 2172509 AA 19950427 CA 1994-2172509 19941013 <--

AU 9479807 A1 19950508 AU 1994-79807 19941013 <--

EP 724432 A1 19960807 EP 1994-930794 19941013

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE

JP 09504027 T2 19970422 JP 1994-512118 19941013

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ST antigen microencapsulation lactide glycolide **microsphere**;

vaccine encapsulation lactide glycolide **microsphere**

IT Vaccines

(microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Antigens

RL: BAC (Biological activity or effector, except adverse); THU

(Therapeutic use); BIOL (Biological study); USES (Uses)

(microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Immunostimulants

(adjuvants, microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Sialoglycoproteins

RL: BAC (Biological activity or effector, except adverse); THU

(Therapeutic use); BIOL (Biological study); USES (Uses)

(gp120env, of **HIV**; microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Virus, animal

(human immunodeficiency, glycoprotein gp120 of; microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Encapsulation

(micro-, microencapsulation of antigens in lactide/glycolide copolymer (**PLGA**) for use as vaccines)

IT Pharmaceutical dosage forms
 (microspheres, microencapsulation of antigens in
 lactide/glycolide copolymer (PLGA) for use as vaccines)
 IT 26780-50-7, DL-Lactide/glycolide copolymer 141256-04-4, QS 21
 RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (microencapsulation of antigens in lactide/glycolide copolymer (

AN 1995:652539 CAPLUS

DN 123:40954

TI Microencapsulation of antigens in lactide/glycolide copolymer (

PLGA) for use as vaccines

IN Cleland, Jeffrey L.; Lim, Amy; Powell, Michael Frank

PA Genentech, Inc., USA

SO PCT Int. Appl., 57 pp.

CODEN: PIXXD2

DT Patent

LA English

FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9511010	A1	19950427	WO 1994-US11753	19941013 <--
	W: AU, CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	CA 2172509	AA	19950427	CA 1994-2172509	19941013 <--
	AU 9479807	A1	19950508	AU 1994-79807	19941013 <--
	EP 724432	A1	19960807	EP 1994-930794	19941013
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 09504027	T2	19970422	JP 1994-512118	19941013
PRAI	US 1993-141796		19931022		
	US 1993-143555		19931025		
	WO 1994-US11753		19941013		

L2 ANSWER 3 OF 20 CAPLUS COPYRIGHT 2002 ACS

AB The successful development of an AIDS vaccine will require formulations that not only invoke the desired immunol. response, but also are stable and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-co-glycolic) acid (PLGA) microspheres was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 mo. In addn., PLGA microspheres contg. the adjuvant, QS21, were also prepd. to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these formulations yielded higher anti-MN rgp120 and anti-V3 loop antibody titers than alum formulations that were administered at higher antigen doses. Different doses of encapsulated MN rgp120 provided a clear and well-defined dose response curve for both anti-MN rgp120 and anti-V3 loop antibody titers. When sol. QS21 was mixed with the encapsulated MN rgp120, the antibody titers were increased by a factor of 5 over the titers with encapsulated MN rgp120 alone. An addnl. five-fold increase in antibody titers was obsd. for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same microspheres. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in PLGA. Furthermore, antibodies induced by these preps. neutralized the MN strain of HIV-1. The neutralization titers for sera from animals immunized with MN rgp120-PLGA and sol. QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and sol. QS21 at the same dose. Overall, these studies validate the in vivo autoboot concept, reveal a method for improving the adjuvant properties of QS21, and indicate the potential of future single shot vaccine formulations.

- TI Development of a single-shot subunit vaccine for **HIV-1**
 SO AIDS Res. Hum. Retroviruses (1994), 10(Suppl. 2), S21-S26
 CODEN: ARHRE7; ISSN: 0889-2229
- AB . . . and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-co-glycolic) acid (**PLGA**) **microspheres** was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 mo. In addn., **PLGA microspheres** contg. the adjuvant, QS21, were also prepd. to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these. . . five-fold increase in antibody titers was obsd. for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preps. neutralized the MN strain of **HIV-1**. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and sol. QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and sol.. . .
- ST vaccine HIV1 polyester **microsphere**
 IT Acquired immune deficiency syndrome
 Vaccines
 (single-shot subunit vaccine for **HIV-1**)
- IT Antigens
 RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (single-shot subunit vaccine for **HIV-1**)
- IT Virus, animal
 (human immunodeficiency 1, single-shot subunit vaccine for **HIV-1**)
- IT Polyesters, biological studies
 RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (hydroxycarboxylic acid-based, single-shot subunit vaccine for **HIV-1**)
- IT Pharmaceutical dosage forms
 (**microspheres**, single-shot subunit vaccine for **HIV-1**)
- IT 34346-01-5, Glycolic acid-lactic acid copolymer
 RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)
 (**microspheres**; single-shot subunit vaccine for **HIV-1**)
- AN 1995:260433 CAPLUS
 DN 122:38660
- TI Development of a single-shot subunit vaccine for **HIV-1**
 AU Cleland, Jeffrey L.; Powell, Michael F.; Lim, Amy; Barron, Lorena; Berman, Phillip W.; Eastman, Donna J.; Nunberg, Jack H.; Wrin, Terri; Vennari, Joann C.
 CS Department of Pharmaceutical Research and Development, Genentech, Inc., San Francisco, CA, 94080, USA
- SO AIDS Res. Hum. Retroviruses (1994), 10(Suppl. 2), S21-S26
 CODEN: ARHRE7; ISSN: 0889-2229
- DT Journal
 LA English
- L2 ANSWER 4 OF 20 CAPLUS COPYRIGHT 2002 ACS
 AB This paper describes the conditions of prepn. of poly(lactide-co-glycolide) **microspheres** with a mean diam. lower than 10 .mu.m obtained by a (w1/o)w2 emulsion solvent evapn. method. Different parameters influencing resp. the size of the inner emulsion and the diam. of the **microspheres** were detd. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in

those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepd. according to the single emulsion solvent evapn. method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aq. phase. Anal. of the release kinetics was carried out in phosphate buffer (PBS), in artificial gastric and intestinal medium. V3 BRU release in PBS was slow, reaching a plateau at 24 h corresponding to 25% of drug release. In addn., V3 BRU was not released in gastric medium within 4 h whereas under the same time conditions, 60% of the drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.

- TI Characterization of V3 BRU peptide-loaded small **PLGA** **microspheres** prepared by a (w1/o)w2 emulsion solvent evaporation method
- SO Int. J. Pharm. (1994), 111(2), 137-45
CODEN: IJPHDE; ISSN: 0378-5173
- AB This paper describes the conditions of prepn. of poly(lactide-co-glycolide) **microspheres** with a mean diam. lower than 10 .mu.m obtained by a (w1/o)w2 emulsion solvent evapn. method. Different parameters influencing resp. the size of the inner emulsion and the diam. of the **microspheres** were detd. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepd. according to the single emulsion solvent evapn. method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aq. phase. Anal. . . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.
- ST polyester V3BRU peptide microencapsulation vaccine; **microsphere** **PLGA** V3BRU peptide vaccine
- IT Solution rate
(peptide release from small poly(lactide-co-glycolide) **microspheres** prepd. by multiple emulsion solvent evapn.)
- IT Peptides, biological studies
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn.)
- IT Vaccines
(prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn. for vaccines)
- IT Virus, animal
(human immunodeficiency, prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn. for vaccines)
- IT Virus, animal
(human immunodeficiency 1, prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn. for vaccines)
- IT Polyesters, biological studies
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(hydroxycarboxylic acid-based, prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn.)

- IT Encapsulation
(micro-, prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn.)
- IT Pharmaceutical dosage forms
(**microspheres**, prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn.)
- IT 26780-50-7, Lactide-glycolide copolymer 159202-27-4
RL: PEP (Physical, engineering or chemical process); THU (Therapeutic use); BIOL (Biological study); PROC (Process); USES (Uses)
(prepn. of peptide-loaded small poly(lactide-co-glycolide) **microspheres** by multiple emulsion solvent evapn.)
- AN 1994:708124 CAPLUS
- DN 121:308124
- TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w1/o)w2 emulsion solvent evaporation method
- AU Prieto, Maria Jose Blanco; Delie, Florence; Fattal, Elias; Tartar, Andre; Puisieux, Francis; Gulik, Annette; Couvreur, Patrick
- CS Laboratoire Physico-Chimie-Pharmacotechnie-Biopharmacie, URA CNRS 1218, Faculte de Pharmacie, 5, Rue Jean Baptiste Clement, Chatenay Malabry, 92296, Fr.
- SO Int. J. Pharm. (1994), 111(2), 137-45
CODEN: IJPHDE; ISSN: 0378-5173
- DT Journal
- LA English
- L2 ANSWER 5 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.
- AB The successful development of an AIDS vaccine will require formulations that not only invoke the desired immunological response, but also are stable and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-coglycolic) acid (**PLGA**) **microspheres** was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 months. In addition, **PLGA microspheres** containing the adjuvant, QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these formulations yielded higher anti-MN rgp120 and anti-V3 loop antibody titers than alum formulations that were administered at higher antigen doses. Different doses of encapsulated MN rgp120 provided a clear and well-defined dose response curve for both anti-MN rgp120 and anti-V3 loop antibody titers. When soluble QS21 was mixed with the encapsulated MN rgp120, the antibody titers were increased by a factor of 5 over the titers with encapsulated MN rgp120 alone. An additional fivefold increase in antibody titers was observed for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preparations neutralized the MN strain of HIV-1. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and soluble QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and soluble QS21 at the same dose. Overall, these studies validate the in vivo autoboot concept, reveal a method for improving the adjuvant properties of QS21, and indicate the potential of future single shot vaccine formulations.
- TI Development of a single-shot subunit vaccine for HIV-1.
- SO AIDS Research and Human Retroviruses, (1994) 10/SUPPL. 2 (S21-S26).
- ISSN: 0889-2229 CODEN: ARHRE7
- AB . . . and easy to administer. A single shot MN rgp120 vaccine

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AN 94369172 EMBASE

DN 1994369172

TI Development of a single-shot subunit vaccine for **HIV-1**.

AU Cleland J.L.; Powell M.F.; Lim A.; Barron L.; Berman P.W.; Eastman D.J.; Nunberg J.H.; Wrin T.; Vennari J.C.

CS Dept. of Pharmaceutical Res./Devt., Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080, United States

SO AIDS Research and Human Retroviruses, (1994) 10/SUPPL. 2 (S21-S26).

ISSN: 0889-2229 CODEN: ARHRE7

CY United States

DT Journal; Conference Article

FS 004 Microbiology

026 Immunology, Serology and Transplantation

037 Drug Literature Index

LA English

SL English

L2 ANSWER 6 OF 20 EMBASE COPYRIGHT 2002 ELSEVIER SCI. B.V.

AB This paper describes the conditions of preparation of poly(lactide-coglycolide) **microspheres** with a mean diameter lower than 10 .mu.m obtained by a (w1/o)w2, emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis of the release kinetics was carried out in phosphate buffer (PBS), in artificial gastric and intestinal medium. V3 BRU release in PBS was slow, reaching a plateau at 24 h corresponding to 25% of drug release. In addition, V3 BRU was not released in gastric medium within 4 h whereas under the same time conditions, 60% of the drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.

TI Characterization of V3 BRU peptide-loaded small **PLGA microspheres** prepared by a (w1/o)w2 emulsion solvent evaporation method.

SO International Journal of Pharmaceutics, (1994) 111/2 (137-145).

ISSN: 0378-5173 CODEN: IJPHDE

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coglycolide) **microspheres** with a mean diameter lower than 10 μm obtained by a (w1/o)w2, emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis. . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.

CT Medical Descriptors:

*physical chemistry
article
biodegradation
drug degradation
drug formulation
drug release
electron microscopy
particle size
partition coefficient
priority journal
*emulsion

microsphere

*peptide
solvent

AN 94285036 EMBASE

DN 1994285036

TI Characterization of V3 BRU peptide-loaded small **PLGA**

microspheres prepared by a (w1/o)w2 emulsion solvent evaporation method.

AU Blanco Prieto M.J.; Delie F.; Fattal E.; Tartar A.; Puisieux F.; Gulik A.; Couvreur P.

CS Lab.Phys.Chim.PharmacoTech./Biophar., URA CNRS 1218, Faculte de Pharmacie, 5, Rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex, France

SO International Journal of Pharmaceutics, (1994) 111/2 (137-145).

ISSN: 0378-5173 CODEN: IJPHDE

CY Netherlands

DT Journal; Article

FS 037 Drug Literature Index

LA English

SL English

L2 ANSWER 7 OF 20 IPA COPYRIGHT 2002 ASHP

AB Poly(lactide-co-glycolide) (polyglactin 370) **microspheres** with a mean diameter less than 10 μm were prepared by a (w1/o)w2 emulsion solvent evaporation method, and parameters influencing **microsphere** size and the entrapment of V3-BRU peptide, a specific immunogenic fraction from GP120 of **HIV**, were determined.

The entrapment efficiency was superior to that of **microspheres** prepared by the single emulsion solvent evaporation method. Electron microscopy demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Peptide release in phosphate buffer was slow, reaching a plateau at 24 h corresponding to 25% drug release. V3-BRU was not released in gastric

medium within 4 h, whereas 60% of the drug was released in intestinal medium.

It was concluded that the polyglactin 370 **microspheres** show potential as an oral adjuvant for **HIV** vaccination.

Ellen Katz Neumann

- TI Characterization of V3-BRU peptide-loaded small **PLGA**
microspheres prepared by a (w1/o)w2 emulsion solvent evaporation
method
- SO International Journal of Pharmaceutics (Netherlands), (Oct 20 1994
) Vol. 111, pp. 137-145. 12 Refs.
CODEN: IJPHDE; ISSN: 0378-5173.

- AB Poly(lactide-co-glycolide) (polyglactin 370) **microspheres**
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size and the entrapment of V3-BRU peptide, a specific immunogenic fraction
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The entrapment efficiency was superior to that of
microspheres prepared by the single emulsion solvent evaporation
method. Electron microscopy demonstrated the presence within the
microspheres of globules corresponding to the w/o initial inner
emulsion in which the peptide was dissolved in the aqueous phase. Peptide.
. . within 4 h, whereas 60% of the drug was released in intestinal
medium.

It was concluded that the polyglactin 370 **microspheres** show
potential as an oral adjuvant for **HIV** vaccination.

Ellen Katz Neumann

- IT Polyglactin 370; **microspheres**; V3-BRU release
- IT V3-BRU; release; polyglactin 370 **microspheres**
- IT Polymers; polyglactin 370; **microspheres**, V3-BRU release
- IT Peptides; V3-BRU; release, polyglactin 370 **microspheres**
- IT Size; **microspheres**; polyglactin 370
- IT Release; V3-BRU; polyglactin 370 **microspheres**
- IT Phosphates; buffers; V3-BRU release, **microspheres**
- IT Buffers; phosphates; V3-BRU release, **microspheres**
- AN 94:14201 IPA
- DN 33-02724
- TI Characterization of V3-BRU peptide-loaded small **PLGA**
microspheres prepared by a (w1/o)w2 emulsion solvent evaporation
method
- AU Blanco Prieto, M. J.; Delie, F.; Fattal, E.; Tartar, A.; Couvreur, P.; et
al
- CS Lab. Physico-Chimie-Pharmacotechnie-Biopharm., URA CNRS 1218, Fac. de
Pharm., 5 rue Jean Baptiste Clement, 92296 Chatenay Malabry Cedex, France
- SO International Journal of Pharmaceutics (Netherlands), (Oct 20 1994
) Vol. 111, pp. 137-145. 12 Refs.
CODEN: IJPHDE; ISSN: 0378-5173.
- DT Journal
- LA English

L2 ANSWER 8 OF 20 LIFESCI COPYRIGHT 2002 CSA

- AB The successful development of an AIDS vaccine will require formulations
that not only invoke the desired immunological response, but also are
stable and easy to administer. A single shot MN rgp120 vaccine formulation
comprised of MN rgp120 encapsulated in poly (lactic-coglycolic) acid (**PLGA**)
microspheres was developed to provide an in vivo
autoboost of antigen. These formulations were designed to yield an in vivo
autoboost at 1, 2, 3 or 4-6 months. In addition, **PLGA**
microspheres containing the adjuvant, QS21, were also prepared to
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titers than alum formulations that were administered at higher antigen doses. Different doses of encapsulated MN rgp120 provided a clear and well-defined dose response curve for both anti-MN rgp120 and anti-V3 loop antibody titers. When soluble QS21 was mixed with the encapsulated MN rgp120, the antibody titers were increased by a factor of 5 over the titers with encapsulated MN rgp120 alone. An additional fivefold increase in antibody titers was observed for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preparations neutralized the MN strain of **HIV-1**. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and soluble QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and soluble QS21 at the same dose. Overall, these studies validate the in vivo autoboot concept, reveal a method for improving the adjuvant properties of QS21, and indicate the potential of future single shot vaccine formulations.

TI Development of a single-shot subunit vaccine for **HIV-1**

SO AIDS RES. HUM. RETROVIRUSES, (1994) vol. 10, no. 2 suppl., pp. S21-S26.

ISSN: 0889-2229.

AB . . . and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-coglycolic) acid (**PLGA**) **microspheres** was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 months. In addition, **PLGA microspheres** containing the adjuvant, QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these . . . fivefold increase in antibody titers was observed for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preparations neutralized the MN strain of **HIV-1**. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and soluble QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and soluble. . .

AN 95:48349 LIFESCI

TI Development of a single-shot subunit vaccine for **HIV-1**

AU Cleland, J.L.; Powell, M.F.; Lim, A.; Barron, L.; Berman, P.W.; Eastman, D.J.; Nunberg, J.H.; Wrin, T.; Vennari, J.C.

CS Dep. Pharm. Res. and Dev. Genentech, Inc., 460 Pt. San Bruno Blvd., South San Francisco, CA 94080, USA

SO AIDS RES. HUM. RETROVIRUSES, (1994) vol. 10, no. 2 suppl., pp. S21-S26.

ISSN: 0889-2229.

DT Journal

FS V

LA English

SL English

L2 ANSWER 9 OF 20 COPYRIGHT 2002 Gale Group

SO High Tech Separations News, (Dec 1994) Vol. 7, No. 7.

ISSN: 1046-039X.

TX A vaccine for **HIV**, the virus that causes AIDS, may still be out of reach, but a promising new controlled-release drug therapy is ready. .

Describing . . . biodegradable depot system, Cleland explained that the

booster compound, in this case QS-21, is encapsulated with a fragment of the **HIV-1** virusthe protein MN rgp120 into solid micro spheres of poly(lactic-coglycolic) acid (**PLGA**). A compound used for over 20 years in surgical sutures, and, more recently, in Lupron Depot, a controlled release medication that treats precocious puberty in children, **PLGA** dissolves slowly, eventually breaking down to lactic and glycolic acids, harmless compounds that occur naturally in the body. The **microspheres** are designed to provide an auto boost release of the QS-21 in one or two month intervals for up to. . .

While designed specifically for use with an **HIV-1** vaccine, Cleland suggested that these combined, encapsulated vaccination preparations could find a number of applications, including infant vaccinations which normally. . .

AN 94:416976 NLDB
 TI Booster' Compound Helps Effectiveness
 SO High Tech Separations News, (Dec 1994) Vol. 7, No. 7.
 ISSN: 1046-039X.
 PB Business Communications Company, Inc
 DT Newsletter
 LA English
 WC 429

L2 ANSWER 10 OF 20 COPYRIGHT 2002 Gale Group

TI **HIV/Vaccine - Microsphere Drug Delivery**
 SO Vaccine Weekly, (14 Nov 1994) .
 ISSN: 1074-2921.
 TX Prieto, M.J.B.; Delie, F.; Fattal, E.; Tartar, A.; Puisieux, F.; Gulik, A.; Couvreur, P. "Characterization of V3 BRU Peptide-Loaded Small **PLGA Microspheres** Prepared by a (w(1)/o)w(2) Emulsion Solvent Evaporation Method." International Journal of Pharmaceutics, October 20, 1994;111(2):137-145.

According . . . abstract of an article published in the International Journal of Pharmaceutics, "This paper describes the conditions of preparation of poly(lactide-coglycolide) **microspheres** with a mean diameter lower than 10 mm obtained by a (w(1)/o)w(2) emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from gp120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis. . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination." The corresponding author for this study is: P Couvreur, Fac Pharm Chatenay Malabry, Phys Chim Pharmacotech Biopharm Lab, CNRS, . . .

AN 94:368410 NLDB
 TI **HIV/Vaccine - Microsphere Drug Delivery**
 SO Vaccine Weekly, (14 Nov 1994) .
 ISSN: 1074-2921.
 PB Charles W Henderson
 DT Newsletter
 LA English

09/586747

WC 309

L2 ANSWER 11 OF 20 COPYRIGHT 2002 Gale Group

TI **HIV/Vaccine - Microsphere** Drug Delivery

SO AIDS Weekly, (14 Nov 1994) .

ISSN: 1069-1456.

TX Prieto, M.J.B.; Delie, F.; Fattal, E.; Tartar, A.; Puisieux, F.; Gulik, A.; Couvreur, P. "Characterization of V3 BRU Peptide-Loaded Small **PLGA Microspheres** Prepared by a (w(1)/o)w(2) Emulsion Solvent Evaporation Method." International Journal of Pharmaceutics, October 20, 1994;111(2):137-145.

According . . . abstract of an article published in the International Journal of Pharmaceutics, "This paper describes the conditions of preparation of poly(lactide-coglycolide) **microspheres** with a mean diameter lower than 10 μ m obtained by a (w(1)/o)w(2) emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from gp120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis. . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination." The corresponding author for this study is: P Couvreur, Fac Pharm Chatenay Malabry, Phys Chim Pharmacotech Biopharm Lab, CNRS, .

AN 94:365631 NLDB

TI **HIV/Vaccine - Microsphere** Drug Delivery

SO AIDS Weekly, (14 Nov 1994) .

ISSN: 1069-1456.

PB CW Henderson, Publisher

DT Newsletter

LA English

WC 309

L2 ANSWER 12 OF 20 COPYRIGHT 2002 Gale Group

TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for **HIV-1**

SO Vaccine Weekly, (12 Sep 1994) .

ISSN: 1074-2921.

TX According . . . high and sustained immunological response with as few injections as possible. A vaccine formulation comprised of MN rgp120 encapsulated in **PLGA microspheres** was developed to provide an in vivo autoboot at 1, 2, 3 or 4-6 months. **PLGA microspheres** containing the adjuvant QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. These formulations yielded higher. . . rgp120 provided a well -defined dose response curve in antibody titers. The addition of soluble or encapsulated QS21 to MN rgp120/**PLGA** greatly enhanced the immune response in both guinea pigs and baboons. These results also indicated the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, the virus neutralization titers induced after one immunization with the encapsulated MN rgp120/QS21 formulation were

equivalent to those obtained. . . immunizations of the soluble MN rgp120/QS21 formulation having significantly higher antigen and QS21 doses. Baboon data also indicated that the **PLGA** formulations provided a longer lasting immune response and higher antibody and virus neutralization titers than soluble QS21 and MN rgp120. . .

AN 94:315589 NLDB

TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for HIV-1

SO Vaccine Weekly, (12 Sep 1994) .

ISSN: 1074-2921.

PB Charles W Henderson

DT Newsletter

LA English

WC 326

L2 ANSWER 13 OF 20 COPYRIGHT 2002 Gale Group

TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for HIV-1

SO AIDS Weekly, (12 Sep 1994) .

TX According . . . high and sustained immunological response with as few injections as possible. A vaccine formulation comprised of MN rgp120 encapsulated in **PLGA microspheres** was developed to provide an in vivo autoboot at 1, 2, 3 or 4-6 months. **PLGA microspheres** containing the adjuvant QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. These formulations yielded higher. . . rgp120 provided a well -defined dose response curve in antibody titers. The addition of soluble or encapsulated QS21 to MN rgp120/**PLGA** greatly enhanced the immune response in both guinea pigs and baboons. These results also indicated the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, the virus neutralization titers induced after one immunization with the encapsulated MN rgp120/QS21 formulation were equivalent to those obtained. . . immunizations of the soluble MN rgp120/QS21 formulation having significantly higher antigen and QS21 doses. Baboon data also indicated that the **PLGA** formulations provided a longer lasting immune response and higher antibody and virus neutralization titers than soluble QS21 and MN rgp120. . .

AN 94:314412 NLDB

TI Immunostimulant/Antiviral. Controlled Release Subunit Vaccine for HIV-1

SO AIDS Weekly, (12 Sep 1994) .

PB CW Henderson, Publisher

DT Newsletter

LA English

WC 326

L2 ANSWER 14 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

AB The successful development of an AIDS vaccine will require formulations that not only invoke the desired immunological response, but also are stable and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-coglycolic) acid (**PLGA**) **microspheres** was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 months. In addition, **PLGA microspheres** containing the adjuvant, QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these formulations yielded higher anti-MN rgp120 and anti-V3 loop antibody titers than alum formulations that were administered at higher antigen doses. Different doses of encapsulated MN rgp120 provided a clear and

well-defined dose response curve for both anti-MN rgp120 and anti-V3 loop antibody titers. When soluble QS21 was mixed with the encapsulated MN rgp120, the antibody titers were increased by a factor of 5 over the titers with encapsulated MN rgp120 alone. An additional fivefold increase in antibody titers was observed for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preparations neutralized the MN strain of **HIV-1**. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and soluble QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and soluble QS21 at the same dose. Overall, these studies validate the in vivo autoboot concept, reveal a method for improving the adjuvant properties of QS21, and indicate the potential of future single shot vaccine formulations.

TI DEVELOPMENT OF A SINGLE-SHOT SUBUNIT VACCINE FOR **HIV-1**

SO AIDS RESEARCH AND HUMAN RETROVIRUSES, (1994) Vol. 10, Supp. 2, pp. S21-S26.

ISSN: 0889-2229.

AB . . . and easy to administer. A single shot MN rgp120 vaccine formulation comprised of MN rgp120 encapsulated in poly (lactic-coglycolic) acid (**PLGA**) **microspheres** was developed to provide an in vivo autoboot of antigen. These formulations were designed to yield an in vivo autoboot at 1, 2, 3 or 4-6 months. In addition, **PLGA microspheres** containing the adjuvant, QS21, were also prepared to provide an in vivo autoboot concomitant with antigen. In guinea pigs, these. . . fivefold increase in antibody titers was observed for guinea pigs immunized with encapsulated MN rgp120 and QS21 on the same **microspheres**. These results suggest that the adjuvant properties of QS21 can be increased by microencapsulation in **PLGA**. Furthermore, antibodies induced by these preparations neutralized the MN strain of **HIV-1**. The neutralization titers for sera from animals immunized with MN rgp120-**PLGA** and soluble QS21 were greater than the titers obtained from guinea pigs that were treated with MN rgp120 and soluble. . .

STP KeyWords Plus (R): RESPONSES; ANTIGEN; **MICROSPHERES**; ADJUVANTS; ASSAY

AN 94:745601 SCISEARCH

GA The Genuine Article (R) Number: PT118

TI DEVELOPMENT OF A SINGLE-SHOT SUBUNIT VACCINE FOR **HIV-1**

AU CLELAND J L (Reprint); POWELL M F; LIM A; BARRON L; BERMAN P W; EASTMAN D J; NUNBERG J H; WRIN T; VENNARI J C

CS GENENTECH INC, DEPT PHARMACEUT RES & DEV, 460 PT SAN BRUNO BLVD, S SAN FRANCISCO, CA, 94080 (Reprint); GENENTECH INC, DEPT IMMUNOL, S SAN FRANCISCO, CA, 94080

CYA USA

SO AIDS RESEARCH AND HUMAN RETROVIRUSES, (1994) Vol. 10, Supp. 2, pp. S21-S26.

ISSN: 0889-2229.

DT Article; Journal

FS LIFE

LA ENGLISH

REC Reference Count: 14

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L2 ANSWER 15 OF 20 SCISEARCH COPYRIGHT 2002 ISI (R)

AB This paper describes the conditions of preparation of poly(lactide-coglycolide) **microspheres** with a mean diameter lower than 10 μ m obtained by a (w(1)/o)w(2) emulsion solvent evaporation method. Different parameters influencing respectively the size of the

inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis of the release kinetics was carried out in phosphate buffer (PBS), in artificial gastric and intestinal medium. V3 BRU release in PBS was slow, reaching a plateau at 24 h corresponding to 25% of drug release. In addition, V3 BRU was not released in gastric medium within 4 h whereas under the same time conditions, 60% of the drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.

TI CHARACTERIZATION OF V3 BRU PEPTIDE-LOADED SMALL **PLGA**

MICROSPHERES PREPARED BY A (W(1)/O)W(2) EMULSION SOLVENT EVAPORATION METHOD

SO INTERNATIONAL JOURNAL OF PHARMACEUTICS, (20 OCT 1994) Vol. 111, No. 2, pp. 137-145.
ISSN: 0378-5173.

AB This paper describes the conditions of preparation of poly(lactide-co-glycolide) **microspheres** with a mean diameter lower than 10 μ m obtained by a (w(1)/o)w(2) emulsion solvent evaporation method. Different parameters influencing respectively the size of the inner emulsion and the diameter of the **microspheres** were determined. V3 BRU, which is a specific immunogenic fraction from GP120 of **HIV**, was encapsulated in those **microspheres**. The entrapment efficiency was shown to be superior to that of **microspheres** prepared according to the single emulsion solvent evaporation method. Electron microscopy observations demonstrated the presence within the **microspheres** of globules corresponding to the w/o initial inner emulsion in which the peptide was dissolved in the aqueous phase. Analysis. . . drug was released in the presence of intestinal medium. These results open up interesting prospects for the use of these **microspheres** as an oral adjuvant for **HIV** vaccination.

ST Author Keywords: POLY(LACTIDE-CO-GLYCOLIDE); BIODEGRADABLE **MICROSPHERE**; MULTIPLE EMULSION; V3 BRU PEPTIDE; ORAL IMMUNIZATION; RELEASE KINETICS

STP KeyWords Plus (R): BIODEGRADABLE **MICROSPHERES**; DELIVERY SYSTEMS; MICROPARTICLES; RELEASE; ACID)

AN 94:622970 SCISEARCH

GA The Genuine Article (R) Number: PH828

TI CHARACTERIZATION OF V3 BRU PEPTIDE-LOADED SMALL **PLGA**

MICROSPHERES PREPARED BY A (W(1)/O)W(2) EMULSION SOLVENT EVAPORATION METHOD

AU PRIETO M J B; DELIE F; FATTAL E; TARTAR A; PUISIEUX F; GULIK A; COUVREUR P (Reprint)

CS FAC PHARM CHATENAY MALABRY, PHYS CHIM PHARMACOTECH BIOPHARM LAB, CNRS, URA 1218, F-92296 CHATENAY MALABRY, FRANCE (Reprint); FAC PHARM CHATENAY MALABRY, PHYS CHIM PHARMACOTECH BIOPHARM LAB, CNRS, URA 1218, F-92296 CHATENAY MALABRY, FRANCE; CHIM BIOMOLEC LAB, CNRS, URA 1309, F-59019 LILLE, FRANCE; CTR GENET MOLEC, UPR A2420, F-91198 GIF SUR YVETTE, FRANCE

CYA FRANCE

SO INTERNATIONAL JOURNAL OF PHARMACEUTICS, (20 OCT 1994) Vol. 111, No. 2, pp. 137-145.
ISSN: 0378-5173.

DT Article; Journal

09/586747

FS LIFE
LA ENGLISH
REC Reference Count: 12
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

L2 ANSWER 16 OF 20 USPATFULL

AB Particles are provided that are not rapidly cleared from the blood stream by the macrophages of the reticuloendothelial system, and that can be modified to achieve variable release rates or to target specific cells or organs. The particles have a core of a multiblock copolymer formed by covalently linking a multifunctional compound with one or more hydrophobic polymers and one or more hydrophilic polymers, and contain a biologically active material. The terminal hydroxyl group of the poly(alkylene glycol) can be used to covalently attach onto the surface of the particles biologically active molecules, including antibodies targeted to specific cells or organs, or molecules affecting the charge, lipophilicity or hydrophilicity of the particle. The surface of the particle can also be modified by attaching biodegradable polymers of the same structure as those forming the core of the particles. The typical size of the particles is between 180 nm and 10,000 nm, preferably between 180 nm and 240 nm, although microparticles can also be formed as described herein. The particles can include magnetic particles or radiopaque materials for diagnostic imaging, biologically active molecules to be delivered to a site, or compounds for targeting the particles. The particles have a prolonged half-life in the blood compared to particles not containing poly(alkylene glycol) moieties on the surface.

PI US 6007845 19991228
WO 9503356 19950202 <--

DETD In a preferred embodiment, a polyester of poly(lactic-co-glycolic) acid (**PLGA**) is used as a hydrophobic erodible polymer bound to the multifunctional compound. These polymers are approved for parenteral administration by the FDA. Because **PLGA** degrades via hydrolysis, in vivo degradation rates can be predicted from in vitro data. **PLGA** degrades to lactic and glycolic acids, substances found naturally in the body. Furthermore, by manipulating the molar ratio of lactic. . .

DETD . . . in ethyl acetate. This copolymer is completely amorphous, which renders it a useful polymer for the fabrication of nanospheres and **microspheres** for controlled release.

DETD . . . to a number of months. Poly-glycolide also has a crystalline structure and a degradation time of one to several months. D,L-**PLGA** is amorphous, with a degradation time in vitro of weeks to months. As the glycolic acid ratio is increased, the. . .

DETD . . . embodiment, a multiblock copolymer is prepared by reacting the terminal group of the hydrophobic polymeric moiety such as PLA or **PLGA** with a suitable polycarboxylic acid monomer, including but not limited to 1,3,5-benzenetricarboxylic acid, butane-1,1,4-tricarboxylic acid, tricarballic acid (propane-1,2,3-tricarboxylic acid), and. . .

DETD In another alternative embodiment, the multiblock copolymer can be blended with a linear hydrophobic-hydrophilic copolymer, for example **PLGA**-PEG mixed with **PLGA** or PLA, prior to fabrication into the particles, to provide different properties on the particles, for example, altering their half-life in vivo. Adding **PLGA**-PEG to other polymers can increase the in vivo half-life of the particles.

DETD . . . from linear copolymers, as shown by ESCA. The amount of PEG (deducted from the ratio between PEG and PLA or **PLGA** comparing C peaks convolution) can be increased from 35.65% to more than 44% using non-linear multiblock copolymers as compared with. . .

DETD . . . diameter can be less than 120 nm. Surprisingly, this is in contrast to particles prepared from linear copolymers, such as PEG-**PLGA** particles, in which the PEG in PEG-**PLGA** particles was able to reduce nanosphere size, as compared to not-coated particles. The composition of the hydrophobic block(s) also affects. . .

DETD . . . toxoid. Examples of organisms from which these antigens are derived include poliovirus, rotavirus, hepatitis A, B, and C, influenza, rabies, **HIV**, measles, mumps, rubella, Bordetella pertussus, Streptococcus pneumoniae, C. diphtheria, C. tetani, Cholera, Salmonella, Neisseria, and Shigella.

DETD The preparation of specific multiblock copolymers of hydrophobic bioerodible polymers such as PLA and **PLGA**, and hydrophilic polyalkylene glycols such as PEG, with multifunctional compounds such as tartaric acid, mucic acid, citric acid, benzene tetracarboxylic. . .

DETD Nanospheres were prepared from a mixture of PEG.sub.3 -citrate-PLA, a **PLGA**-PEG copolymer and a polycaprolactone homopolymer in a ratio of 1:1:3 by weight, using an emulsion/evaporation technique as described above. The. . .

DETD . . . in vitro over several hours, but have different release kinetics. The molecular weight does not effect the release pattern of PEG-**PLGA** nanospheres, since the drug is completely released in about ten hours using copolymers with a PEG m.w. of 5, 12, . . .

DETD Polymer degradation kinetics were also investigated in vitro. With PEG-**PLGA**, PEG-PCL and (PEG).sub.3 -PLA particles, the polymers start to degrade after weeks. Nanosphere cores made of polyanhydrides start to degrade. . .

DETD The amount of drug loading can have a strong effect on the release kinetics. PEG-**PLGA** nanospheres containing 33% wt of lidocaine can release the drug for over 12 hours. Surprisingly, particles loaded with 10% of. . .

AN 1999:170238 USPATFULL

TI Nanoparticles and microparticles of non-linear hydrophilic-hydrophobic multiblock copolymers

IN Domb, Abraham J., Efrat, Israel
Gref, Ruxandra, Nancy, France
Minamitake, Yoshiharu, Gumma, Japan
Peracchia, Maria Teresa, Parma, Italy
Langer, Robert S., Newton, MA, United States

PA Massachusetts Institute of Technology, Cambridge, MA, United States (U.S. corporation)

PI US 6007845 19991228
WO 9503356 19950202 <--

AI US 1996-582993 19960325 (8)
WO 1994-US8287 19940722
19960122 PCT 371 date
19960122 PCT 102(e) date

DT Utility

FS Granted

EXNAM Primary Examiner: Smith, Lynette R. F.; Assistant Examiner: Lee, Datquan

LREP Arnall Golden & Gregory, LLP

CLMN Number of Claims: 38

ECL Exemplary Claim: 1

DRWN 12 Drawing Figure(s); 7 Drawing Page(s)

LN.CNT 1368

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 17 OF 20 USPATFULL

AB A composition for delivering an active agent to the lymphatic system comprises a plurality of colloidal particles and an active agent associated with each particle, wherein the surface of each particle has

a hydrophobicity ratio of less than 10 as defined by hydrophobic interaction chromatography.

PI US 5792475 19980811
WO 9402122 19940203 <--

SUMM . . . of malignant diseases. This subject has been reviewed extensively by S. E. Strand and others (L. Bergquist et al. in **Microspheres** and Drug Therapy, Immunological and Medical Aspects p.263 (Edited by Davis et al.) Elsevier 1984).

SUMM The particles can be non-biodegradable. The biodegradable particles suitably include **microspheres** and nanoparticles, microcapsules or nanocapsules, emulsions, microemulsions, liposomes and mimics of lipoproteins and chylomicrons. Suitable materials for these include polylactic. . .

SUMM Poly(D,L-lactide-co-glycolide) or **PLGA** (75:25, Mw.sub.GPC 63kD) was purchased in the form of Resomer.RTM. RG755 from Boehringer Ingelheim (Ingelheim, Germany). The poly(.beta.-malic acid-co-benzyl malate) (PMLABe.sub.100-x. . .

SUMM The copolymer (**PLGA**, PMLABe.sub.100, PMLABe.sub.90 H.sub.10, or PMLABe.sub.80 H.sub.20) was dissolved in acetone (10 ml, 20.0 mg/ml for **PLGA** or 5.0 mg/ml for PMLABeH) and a mixture of deionized water and ethanol (1.1) was added dropwise (25 G syringe. . .

SUMM A suitable particle with a grafted modifying agent would be for example human serum albumin **microspheres** in the size region 80-140 nm with a grafted surface PEG chain. These can be prepared by precipitating a 2% solution of human serum albumin **microspheres** in the size region 80-nm with a grafted surface PEG chain. These can be prepared by precipitating a 2% solution of human serum albumin-polyethylene glycol co-polymer in a water/acetone mixture with ethyl acetate during stirring. The **microspheres** are crosslinked with glutaraldehyde. Alternatively human serum albumin microspheres with surface grafted PEG chains can be produced by mixing a. . .

SUMM (iii) The delivery of drugs to lymph nodes using carriers such as **microspheres**, microcapsules, emulsions, liposomes. Agents and diseases relevant in this regard include antimicrobial agents for treatment of infection of the nodes such as in filariasis, brucellosis, tuberculosis **HIV**, antitumour agents such as mitomycin C, bleomycin, etc. or antibodies against tumours and macrophage modifying agents such as interferons, MDP,. . .

DETD of drainage of small particles from a subcutaneous injection site. Certain materials increases the sequestration of polystyrene **microspheres** in the lymph nodes by surprising amount in comparison to uncoated particles and those coating agents previously described in the. . .

AN 1998:95252 USPTFULL

TI Lymphatic delivery composition

IN Davis, Stanley S., Nottingham, United Kingdom
Illum, Lisbeth, Nottingham, United Kingdom
Christy, Nicola, Nottingham, United Kingdom
Moghimi, Moein, Nottingham, United Kingdom

PA Danbiosyst UK Limited, Nottingham, United Kingdom (non-U.S. corporation)

PI US 5792475 19980811
WO 9402122 19940203 <--

AI US 1995-374671 19950414 (8)
WO 1993-GB1596 19930728
19950414 PCT 371 date
19950414 PCT 102(e) date

PRAI GB 1992-16082 19920728

DT Utility

FS Granted

EXNAM Primary Examiner: Clardy, S. Mark; Assistant Examiner: Harrison, Robert

H.

LREP Arnall, Golden & Gregory, LLP
 CLMN Number of Claims: 20
 ECL Exemplary Claim: 1
 DRWN 5 Drawing Figure(s); 4 Drawing Page(s)
 LN.CNT 1139
 CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 18 OF 20 USPATFULL

AB Methods for excipient stabilization of dry or aqueous polypeptides treated with organic solvents are disclosed, wherein the polypeptide is admixed with trehalose, a polyol having a molecular weight less than about 70,000 kD.

PI US 5589167 19961231

WO 9419020 19940901

<--

SUMM Polypeptides of interest include glycosylated and unglycosylated polypeptides, such as growth hormone, the interferons, and viral proteins such as **HIV** protease and gp120.

SUMM . . . 22:547-556 [1983]), non-degradable ethylenevinyl acetate (Langer, et al., supra), degradable lactic acid-glycolic acid copolymers such as the Lupron Depot.TM. (injectable **microspheres** composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid (EP 133,988). While polymers such as ethylene-vinyl acetate. . .

DETD . . . for this application was a copolymer of lactic and glycolic acids which is often referred to as poly(lactic/glycolic acid) or **PLGA**. To incorporate hGH into this polymer, the **PLGA** must be dissolved in a water immiscible solvent. The most commonly used solvent for dissolution of **PLGA** has been methylene chloride which provides both water immiscibility and **PLGA** solubility.

DETD In general, for production of hGH-**PLGA microspheres**, the polypeptide was added to a solution of methylene chloride containing **PLGA**. In initial studies, the polypeptide was added in the form of a milled lyophilized powder. After polypeptide addition, the methylene. . . was added to an emulsification bath. This process resulted in the extraction of methylene chloride with the concomitant formation of **PLGA microspheres** containing hGH. The polypeptide released from these **microspheres** was then studied to determine the integrity of hGH after incorporation into the **microspheres**. Assessment of released hGH was performed by analytical size exclusion chromatography (SEC-HPLC) as well as other techniques. Size exclusion chromatography indicated that hGH was released from the **PLGA microspheres** in the form of the native monomer, aggregates, and an unknown structure which eluted between the monomer and dimer. The. . .

DETD The release of monomeric native hGH from the **PLGA microspheres** is required for a successful long acting formulation. Previous studies investigated several organic solvents as alternatives to methylene chloride. This. . . hGH was susceptible to damage by several organic solvents. Since methylene chloride provided the desired solvent properties (i.e. water immiscibility, **PLGA** dissolution, etc.) for **PLGA microsphere** production and other solvents did not significantly improve hGH stability, methylene chloride was chosen for the production of the **PLGA microspheres**. The polypeptide used for the solvent study and in the **PLGA** production process was formulated and lyophilized in ammonium bicarbonate buffer at pH 7. Therefore, this study was performed to develop formulations which would stabilize hGH during the production of the **PLGA microspheres**.

DETD . . . for 30 seconds in a 47 kHz bath sonicator (Cole Parmer, Model

08849-00) to simulate the homogenization step in the **microsphere** production process. If the formulation stabilized hGH against denaturation in this test, it was further assessed by homogenization in methylene. . . .

DETD To increase the amount of polypeptide loaded into the **microspheres**, the amount of excipient should be minimized. Therefore, lower concentrations of mannitol (2 and 5 mg/ml) with 10 mg/ml hGH. . . .

DETD Microencapsulation of proteins in biodegradable polymers often requires the use of organic solvents to solubilize the polymer. The polymer, typically **PLGA**, polylactide (PLA), or polyglycolide (PGA), is first dissolved in an organic solvent that is not completely miscible with water. The. . . .

DETD . . . than 0.5 mL of drug per gram of polymer typically result in a large initial burst of drug from the **microspheres**. To avoid these difficulties, a solid drug formulation can be used in place of the aqueous drug solution. Thus, a solid-in-oil-in-water process can be used to produce **microspheres** with high drug loading (greater than 10%) with low to moderate initial bursts.

DETD . . . for microencapsulation must be stable in organic solvents and it must have a small size (1-5 .mu.m) relative to the **microspheres** (30-100 .mu.m) to permit high loading and low burst of the drug. For protein formulations, one method of obtaining small. . . .

DETD . . . microencapsulation in a polymer matrix since it can provide a high loading (able to pack more solid into 30-100 .mu.m **microspheres**) of homogeneously dispersed solid protein (reduced burst due to fine suspension).

AN 96:120585 USPATFULL

TI Excipient stabilization of polypeptides treated with organic solvents

IN Cleland, Jeffrey L., San Carlos, CA, United States

Jones, Andrew J. S., San Mateo, CA, United States

PA Genentech, Inc., South San Francisco, CA, United States (U.S. corporation)

PI US 5589167 19961231

WO 9419020 19940901

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AI US 1994-256187 19940408 (8)

WO 1994-US1666 19940217

19940408 PCT 371 date

19940408 PCT 102(e) date

RLI Continuation-in-part of Ser. No. US 1993-21421, filed on 23 Feb 1993, now abandoned

DT Utility

FS Granted

EXNAM Primary Examiner: Sayala, Chhaya D.

LREP Fitts, Renee A., Torchia, Timothy E.

CLMN Number of Claims: 16

ECL Exemplary Claim: 1

DRWN No Drawings

LN.CNT 912

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 19 OF 20 USPATFULL

AB This invention is directed to oral parenteral and intestinal vaccines and eir use against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres**.

TI Vaccines against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres**

PI US 5417986 19950523 <--

AB . . . to oral parenteral and intestinal vaccines and eir use against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres**.

SUMM This invention relates to parenteral and oral-intestinal vaccines against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres** (matrix).

SUMM . . . overcoming these problems is to homogeneously disperse the antigen of interest within the polymeric matrix of appropriately sized biodegradable, biocompatible **microspheres** that are specifically taken up by GALT. Eldridge et. al. have used a murine model to show that orally-administered 1-10 micrometer **microspheres** consisting of polymerized lactide and glycolide, (the same materials used in resorbable sutures), were readily taken up into Peyer's patches, and the 1-5 micrometer size were rapidly phagocytized by macrophages. **Microspheres** that were 5-10 micrometers (microns) remained in the Peyer's patch for up to 35 days, whereas those less than 5. . .

SUMM . . . the immunogenicity of antigens that contact the intestinal mucosa, applicants investigated the effect of homogeneously dispersing AF/RI pili within biodegradable **microspheres** that included a size range selected for Peyer's Patch localization. New Zealand White rabbits were primed twice with 50 micrograms. . .

DRWD FIG. 1 shows the size destribution of **microspheres** wherein the particle size distribution (%) is (a) By number 1-5 (91) and 6-10 (9) and Co) By weight 1-5. . .

DRWD FIG. 2 shows a scanning electron micrograph of **microspheres**.

DRWD . . . mononuclear cells in vitro producing a primary IgM antibody response specific to AF/RI. Immunization with antigen encapsulated in biodegradable, biocompatible **microspheres** consisting of lactide/glycolide copolymers has been shown to endow substantially enhanced immunity over immunization with the free antigen. To determine. . . with free AF/RI in a dose range from 15 to 150 ng/ml or with equivalent doses of AF/RI contained in **microspheres**. Supernatants were harvested on days 7, 9, 12, and 14 of culture and were assayed for free AF/RI pilus protein. . .

DRWD . . . mononuclear cells in vitro producing a primary IgM antibody response specific to AF/RI. Immunization with antigen encapsulated in biodegradable, biocompatible **microspheres** consisting of lactide/glycolide copolymers has been shown to endow substantially enhanced immunity over immunization with the free antigen. To determine. . . with free AF/RI in a dose range from 15 to 150 ng/ml or with equivalent dose of AF/RI contained in **microspheres**. Supernatants were harvested on days 7, 9, 12, and 14 of culture and were assayed for free AF/RI pilus protein. . .

DRWD . . . RDEC-1 to attach to rabbit intestinal brush borders. We investigated the immunopotentiating effect of encapsulating purified AF/RI into biodegradable non-reactive **microspheres** composed of polymerized lactide and glycolide, materials used in resorbable sutures. The **microspheres** had a size range of 5-10 microns, a size selected for Peyer's Patch localizaiton, and contained 0.62% protein by weight.. . proliferation in responce to purified AR/RI was conducted in vitro at seven days and showed that encapsulating the antigen into **microspheres** enhanced the cellular immune response in the Peyer's Patch; however, no significant increase was observed in spleen or mesenteric lymph. . .

DRWD . . . cell epitope. We used these peptides to investigate a possible immunopotentiating effect of encapsulating purified Af/RI pili into biodegradable, biocompatible **microspheres** composed of polymerized lactide and glycolide at a size range that promotes

localization in the Peyer's Patch (5-10 micrometers). NZW. . . .

DRWD . . . was to determine if AR/R1 pilus protein immune response is enhanced by microencapsulation. The AF/R1 was incorporated into biodegradable, biocompatible **microspheres** composed of lactide-glycolide copolymers, had a size range of 5-10 micrometer and containing 0.62% pilus protein by weight. Initially, NZW. . . . predicted epitopes were similar to those obtained with purified AF/R1. In conclusion, intestinal immunization with AF/R1 pilus protein contained within **microspheres** greatly enhances both the spleen and Peyer's patch B-cell responses to predicted T & B-cell epitopes.

DRWD FIG. 25 shows that rabbits numbers 21 and 22 received intraduodenal administration of AF/R1 **microspheres** at doses of AF/R1 of 200 micrograms (ug) on day 0 and 100 ug on day 7, 14, and 21. . . .

DRWD FIG. 29. Particle size distribution of CFA/II **microsphere** vaccine Lot L74F2 values are percent frequency of number or volume verses distribution. Particle size (diameter) in microns. 63% by. . . .

DRWD FIG. 30. Scanning electron photomicrograph of CFA/II **microsphere** vaccine Lot L7472 standard bar represents 5 um distance.

DRWD FIG. 31. Twenty-two hour CFA/II release study of CFA/II **microsphere** vaccine Lot L7472. Percent cumulative release of CFA/II from three sample: A, 33.12 mgm; B, 29.50 mgm c, 24.20 mgm. . . .

DRWD FIG. 32. Serum IgG antibody reponse to CFA/II **microsphere** vaccine Lot L7472 following 2 25 ug protein IM immunization on day 0 in 2 rabbits. Antibody determines on serial. . . .

DRWD FIG. 33. Serum IgG antibody response to CFA/II **microsphere** vaccine Lot L7F2 following 2 25 ug protein IM immunizations on day 0 if rabbit 107 & 109. Antibody determined. . . .

DRWD . . . (FIG. 34(b)), 83 (FIG. 34(c)), 86 (FIG. 34(d)), and 87 (FIG. 34(e)) immunized intraduodenally with 50 mgm protein of CFA/II **microsphere** vaccine 4 and 7 days earlier. The cells are challenged in vitro with CFA/II or BSA at 500, 50 and. . . .

DRWD . . . (FIG. 35(b)), 80 (FIG. 35(c)), 88 (FIG. 35(d)), and 91 (FIG. 35(e)) immunized intraduodenally with 50 mgm protein of CFA/II **microspheres** vaccine 14 and 7 days earlier. The cells are challenged in vitro with CFA with CFA/II or BSA at 500,. . . .

DRWD . . . (FIG. 36(b)), 83 (FIG. 36(c)), 86 (FIG. 36(d)), and 87 (FIG. 36(e)) immunized intraduodenally with 50 mgm protein of CFA/II **microsphere** vaccine 14 and 7 days earlier. These were cells placed into microculture and tested on day 0, 1, 2, 3,. . . .

DRWD FIGS. 11 and 12 serve to illustrate that inclusion of Escherichia coli pilus antigen in **microspheres** enhances cellular immunogenicity.

DRWD . . . gastrointestinal tract (GI) transit and to target immunoresponsive tissue. We tested the effect of incorporating AF/R1 pilus antigen into resorbable **microspheres** upon its ability to induce primary mucosal and systemic antibody responses after direct inoculation into the GI tract.

DRWD Rabbits were inoculated with 50 micrograms of AF/R1 pilus antigen alone or incorporated into uniformly sized (5-10 microns) resorbably **microspheres** (MIC) of poly(DL-lactide-coglycolide). Inoculation was by intra-duodenal (ID) intubation via endoscopy or directly into the ileum near a Peyer's patch. . . .

DRWD . . . immunogenic as shown by measurable mucosal and some strong serum responses. It must be determined whether priming with antigen in **microspheres** can enhance secondary responses.

DRWD . . . partial listing of these viruses (including any derivative thereof) include hepatitis A, hepatitis B, rotaviruses, polio virus human immunodeficiency viruses (HIV), Herpes Simplex virus type 1 (cold sores), Herpes Simplex virus type 2 (Herpesvirus genitalis), Varicella-zoster virus (chicken pox, shingles),

Epstein-Barr. . . .

DRWD A. (1) To homogeneously disperse antigens of enteropathic organisms within the polymeric matrix of biocompatible and biodegradable **microspheres**, 1 nanogram (ng) to 12 microns in diameter, utilizing equal molar parts of polymerized lactide and glycolide (50:50 DL-PLG, i.e.. . . to 52:48 DL-PLG) such that the core load is within the range of about 0.1 to 1.5% by volume. The **microspheres** containing the dispersed antigen can then be used to immunize the intestine to produce a humoral immune response composed of. . . .

DRWD . . . thus promoting colonization resulting in diarrhea. AF/R1 pilus protein was homogeneously dispersed within a polymeric matrix of biocompatible and biodegradable **microspheres**, 1-12 microns in diameter (FIG. 1 and photograph 1) using equal molar parts of polymerized lactide and glycolide (50:50 DL-PLG). . . .

DRWD (3) The **microspheres** were found to contain immunogenic AF/R1 by immunizing both rabbit spleen (FIG. 2) and Peyer's patch (FIG. 3) B-cells in. . . .

DRWD (4) **Microspheres** containing 50 micrograms of AF/R1 were used to intraintestinally (intraduodenally) immunize rabbits on two separate occasions 1 week apart. One. . . .

DRWD B. **Microspheres** do not have to be made up just prior to use as with liposomes. Also liposomes have not been effective. . . .

DRWD C. (1) Only a small amount of antigen is required (ugs) when dispersed within **microspheres** compared to larger amounts (mgms) when antigen is used alone for intestinal immunization.

DRWD (2) Antigen dispersed within **microspheres** can be used orally for intestinal immunization whereas antigen alone used orally even with gastric acid neutralization requires a large. . . .

DRWD (3) Synthetic peptides with and without attached synthetic adjuvants representing peptide fragments of protein antigens can also be dispersed within **microspheres** for oral-intestinal immunization. Free peptides would be destroyed by digestive processes at the level of the stomach and intestine. Any. . . .

DRWD (4) **Microspheres** containing antigen maybe placed into gelatin-like capsules for oral administration and intestinal release for improved intestinal immunization.

DRWD (5) **Microspheres** promote antigen uptake from the intestine and the development of cellular immune (T-cell and B-Cell) responses to antigen components such. . . .

DRWD (6) The development of intestinal T-cell responses to antigens dispersed within **microspheres** indicate that T-cell immunological memory will be established leading to long-lived intestinal immunity. This long-lived intestinal immunity (T-cell) is very. . . .

DRWD (2) **Microspheres** containing adherence pilus protein AF/R1 or its antigen peptides for oral intestinal immunization of rabbits against RDEC-1 infection.

DRWD (4) **Microspheres** containing adherence pilus proteins CFA/I, II, III and IV or their antigen peptides for oral intestinal immunization of humans against. . . .

DRWD (2) By using the **microspheres**, we are now able to immunize the intestine of animals and man with antigens not normally immunogenic for the intestinal. . . .

DRWD (3) Establishing long-lived immunological memory in the intestine is now possible because T-cells are immunized using **microspheres**.

DRWD (4) Antigens that can be dispersed into **microspheres** for intestinal immunization include the following: proteins, glycoproteins, synthetic peptides, carbohydrates, synthetic polysaccharides, lipids, glycolipids, lipopolysaccharides (LPS), synthetic lipopolysaccharides and. . . .

DRWD . . . can be directed to either systemic (spleen and serum antibody)

or local (intestine, Peyer's patch) by the size of the **microspheres** used for the intestinal immunization.

Microspheres 5-10 microns in diameter remain within macrophage cells at the level of the Peyer's patch in the intestine and lead to a local intestinal immune response. **Microspheres** 1 μ m--5 microns in diameter leave the Peyer's patch contained within macrophages and migrate to the mesenteric lymph node and. . .

DRWD . . . antibody mediated adverse reactions because of preexisting antibody especially cytophilic or IgE antibody may be minimized or eliminated by using **microspheres** because of their being phagocytized by macrophages and the antigen is only available as being attached to the cell surface. . .

DRWD (7) Immunization with **microspheres** containing antigen leads to primarily IgA and IgG antibody responses rather than an IgE antibody response, thus preventing subsequent adverse. . .

DRWD . . . Briefly, equal molar parts of DL-lactide and glycolide were polymerized and then dissolved to incorporate AF/R1 into spherical particles. The **microspheres** contained 0.62% protein by weight and ranged in size from 1 to 12 micrometers. Both the microencapsulated and non-encapsulated AF/R1. . .

DRWD . . . inserted through the biopsy channel and threaded 2-3 cm into the small intestine. Inoculums of pili or pili embedded in **microspheres** were injected through the catheter into the duodenum and the endoscope was withdrawn. Animals were monitored daily for signs of. . .

DRWD . . . Peyer's patch cells following intraduodenal inoculation of antigen which had been homogeneously dispersed into the polymeric matrix of biodegradable, biocompatible **microspheres**. The immunopotentiating effect of encapsulating purified AF/R1 pili as a mucosal delivery system may be explained by one or more. . . (c) Once inside the Peyer's patch, microencapsulation appears to facilitate the rapid phagocytosis of the antigen by macrophages, and the **microspheres** which are 5-10 micrometers become localized within the Peyer's patch. (d) Microencapsulation of the antigen may improve the efficiency of. . . food antigens, but they are antigenic because of the bacterial context in which they are presented. The particulate nature of **microspheres** may serve to mimic that context. It may be important to note that we also observed a significant response to. . .

DRWD The **microspheres** used in these experiments included a size range from 1 to 12 micrometers. The 1 to 5 micrometer particles have. . . spleen may reflect priming of MLN or splenic lymphocytes by antigen-presenting/accessory cells which have phagocytosed 1 to 5 micrometer antigen-laden **microspheres** in the Peyer's patch and then disseminated onto the MLN. Alternatively, these responses may be a result of the normal. . .

DRWD . . . without requiring carrier molecules or adjuvants which may complicate vaccine production or delay regulatory approval. The incorporation of antigen into **microspheres** appears to provide an ideal mucosal delivery system for oral vaccine immunogens because the observed immunopotentiating effect is achieved without. . .

DRWD . . . initiate a mucosal response but is susceptible to digestion in the gut. The incorporation of AF/R1 into biocompatible, nondigestible **microspheres** enhanced mucosal cellular immune responses to RDEC-1. We have demonstrated that immunization with AF/R1 Pili in **microspheres** protect rabbits against infection with RDEC-1.

DRWD Six rabbits received intra-duodenal immunization of AF/R1 **microspheres** (0.62% coreloading by weight) at 200 μ g AF/R1 on day 0 then boosted with 100 μ g AF/R1 in **microspheres** on days 7, 14, and 21 followed RDEC-1 challenge with $10^{8.8}$ organisms one week

latter than observed for 1 week. . . infection and strongly indicates similar results should be expected with enterotoxigenicity E. coli using the Colony Forming Antigens (CFA's) in **microsphere** vaccines.

DRWD . . . we showed potentiation of the mucosal cellular immune response to the AF/R1 pilus of RDEC-1 by incorporation into biodegradable polylactide-coglycolide **microspheres** (AF/R1-MS). We now present efficacy testing of this vaccine. Six rabbits were primed with 200 ug and boosted with 100. . .

DRWD More recently, applicants have focused on areas of this invention related to an immunostimulating composition comprising encapsulating **microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** are comprised of (a) a biodegradable-biocompatible poly (DL-lactide-co-glycolide) as the bulk matrix, wherein the relative ratio between the amount of. . .

DRWD 1. An immunostimulating composition comprising encapsulating-**microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a biodegradable-biocompatible poly (DL-lactide-co-glycolide) as. . .

DRWD 4. An immunostimulating composition according to paragraph 2 wherein the size of more than 50% of said **microspheres** is between 5 to 10 um in diameter by volume.

DRWD . . . sum, the Colony Factor Antigen (CFA/II) from enterotoxigenic E coli (ETEC) prepared under GMP was successfully incorporated into biodegradable polymer **microspheres** (CFA/II BPM) under GMP and found to be safe and immunogenic when administered intra-duodenally to rabbits. CFA/II was incorporated into poly (D,L-lactide-co-glycolide) (**PLGA**) **microspheres** which were administered by direct endoscopy into the duodenum. Following vaccination, Peyer's patchcells responded by lymphocyte proliferation to in vitro. . .

DRWD . . . shown to be safe in a variety of applications in human beings and in animals (28-32). Delivery of antigens via **microspheres** composed of biodegradable, biocompatible lactide/glycolide polymers (29-32) may enhance the mucosal response by protecting the antigen from digestion and targeting. . . them to lymphoid cells in Peyer's patches (29-32). McQueen et al. (33) have shown that E. coli AF/R1 pili in **PLGA microspheres**, introduced intra-duodenally in rabbits, protected them against diarrhea and weight loss when challenged with the parent strain rabbit diarrheagenic strain. . .

DRWD In order to improve the CFA/II vaccine it was incorporated into **PLGA microspheres** under GMP in order to protect it from digestion and target it to the intestinal lymphoid system. The CFA/II BPM. . .

DRWD CFA/II Biodegradable Polymer **Microspheres**

DRWD About 1 mgm of **microspheres** were dispersed in 2 ml of 1% Polysorbate 60.degree. (Ruger Chemical Co. Inc. Irvington, N.J.) in water in a 5. . . observed under a calibrated optical microscope with 43.times. magification. Using a precalibrated eye-piece micrometer, the diameter of 150 randomly chosen **microspheres**, was determined and the **microsphere** size distribution was determined

DRWD **Microspheres** were sprinkled on the surface of 10 mm stub covered with a non-conductive adhesive (Sticky-Tab, Ernest F. Fullem, Inc., Lutham,. . .

DRWD Preparation Of CFA/II **Microspheres**

DRWD Solvent extraction technique was used to encapsulate the freeze dried CFA/II into poly(lactide-co-glycolide) (Medisorb Technologies International, viscosity 0.73 dl/g) **microspheres** in the 1-10 um size range to achieve theoretical antigen loading of 1% by weight. The freeze dried antigen-sugar & matrix was dispersed in an acetolnitrile solution of the polymer and then emulsified to achieve desired droplet

size. **Microspheres** were solidified and recovered by using heptane as extracting solvent. The **microsphere** batches were pooled and vacuum dried to remove traces of solvent.

DRWD Protein Content The CFA/II **microspheres** were dissolved in 0.9% SDS in 0.1N NaOH for 18 hr with stirring then neutralized to pH 7 and assayed.. . .

DRWD One hundred and fifty mgm of CFA/II **microspheres** were dissolved in 3 ml of acetonitrile by sonication for 3 hours. One ml sample was injected into a Karl. . .

DRWD Ten mgm of CFA II **microspheres** were dissolved in 1 ml DMF then analysed using gas chromatography and comparing peak heights to external standards of either acetonitrile or heptane diluted in DMF with 10 mgm of blank **microspheres**. The results are expressed as percent by weight.

DRWD One hundred mgm of CFA/II **microsphere**(single dose) are suspended in 2 ml of sterile saline than poured into 2 blood agar plates (1 ml each). All. . . are counted and identified after 48 hours in culture at 37.degree. C. and expressed as total number. Similiar amount of **microspheres** is in 0.25 ml aliquots poured onto 4 different fungal culture plates (Sabhiragar, casein peptone agar with chloramphenicol, brain heart. . .

DRWD CFA/II Release From **Microsphere** Study

DRWD Two doses of one hundred mgm CFA/II **microspheres** were suspended by sonication for 5 minutes in 3.1 mls of sterile vaccine diluent consisting of injectable saline containing 0.5%. . .

DRWD Two Rabbits were immunized with CFA/II **microsphere** vaccine at 25 ug protein in two different sites intra-muscularly on day 0. Sera were obtained from all animals before. . .

DRWD Rabbits (N=5) were vaccinated with CFA/II **microspheres** containing either 25 or 50 ug of protein suspended in 1 ml of PBS containing 0.5% Polysorbate 60.RTM. on day 0 and 7 by sonication. The **microspheres** were injected through an Olympus BF type P10 bronchoscope into the duodenum of the rabbits following sedation with an intra. . . catheter passed through the biospy channel. The catheter was advanced through the pylorus 3-4 cm into the duodenum and the **microsphere** suspension in 1 ml of PBS was injected, followed by a 9 ml flush of PBS and removal of the. . .

DRWD Spleen cells were obtained from immunized rabbits on day 14 following intra-duodenal immunization with CFA/II **microsphere** vaccine. The cells were placed in 96 well round bottom microculture plate at a final concentration of 6.times.10.sup.5 cells/well and. . .

DRWD The results of size frequency analysis of 150 randomly chosen **microspheres** are shown in (FIG. 29). The particle size distribution is plotted in % frequency against particle size in diameter (size). . .

DRWD The **microspheres** are seen in (FIG. 30) which is a scanning electron photomicrograph. Nearly all the **microspheres** are less than 10 um as compared to the 5 um bar. Also the surfaces of the **microsphere** are smooth and demonstrate lack of pores.

DRWD . . . 1.232%.+-0.13 SD; and K65A8, 0.966%.+-0.128 SD. The mean average protein load is 1.16%.+-0.15 SD. The protein load of the CFA/II **microsphere** vaccine in the final dose vial is the following: Lot L74F2, 1.175%.+-0.17SD.

DRWD The CFA/II **microsphere** vaccine (Lot 74F2) percent water content was found using the Karl Fischer titrimeter method to be 2.154% using triplicate samples.

DRWD The acetonitrile residuals of the 4 individual CFA/II **microsphere** batches are the following: K62A8, <0.1%; K62A8, <0.1%, K64A8, <0.1%; and K65A8, <0.1%. The acetonitrile residual of the CFA/II **microsphere** vaccine in the final dose vial is the

following: Lot L74F2, 0.07. \pm 0.05%. The heptane residual of the 4 individual CFA/II **microsphere** batches are the following: K62A8, 1.9%; K63A8, 1.4%; K64A8, 1.6% and K65A8, 1.6%. Following pooling in heptane and subsequent drying, the heptane residual of the CFA/II **microsphere** vaccine in the final dose vial is the following: Lot L74F2, 1.6. \pm 0.1%.

DRWD One hundred milligrams (a single dose) of CFA/II **microsphere** vaccine (Lot L74F2) in the final dose vial was suspended in a 2 ml of sterile saline and 1 ml. . . as a micrococcus species. All these bacteria are considered to be nonpathogenic to humans. An additional 100 mgms of CFA/II **microsphere** vaccine (Lot L74F2) were suspended in 2 ml of sterile saline and 0.25 ml poured onto four different fungal culture. . . .

DRWD CFA Release From **Microsphere** Study

DRWD Two one hundred milligrams (a single dose) of CFA/II **microsphere** vaccine in the final dose vials were suspended in 3.1 mls of the sterile diluent consisting of 0.85N saline prepared. . . .

DRWD . . . The mice gained an average of 2.3 gms and the guinea pigs gained an average of 43 grams. The CFA/II **microsphere** vaccine therefore passed the general safety test.

DRWD Two rabbits were immunized in two separate sites intra-muscularly with 25 ug of protein of CFA/II **microsphere** vaccine (Lot L74F2) in the final dose vial. Sera samples were obtained before and 7 and 14 days following immunization. . . .

DRWD Five rabbits were immunized intra-duodenally with CFA/II **microspheres** containing either 25 ug of protein (human dose equivalent) or 50 ug of protein on days 0 and 7 and. . . .

DRWD Five rabbits immunized intraduodenally with CFA/II **microsphere** containing 50 ug of CFA/II protein at days 0, 7 then sacrificed at day 14 were studied. The spleen cells. . . .

DRWD McQueen et al (33) has found that the AF/R1 adhesin of rabbit diarrheagenic Escheria coli (RDEC-1) incorporated into biodegradable **microspheres** could function as a safe and effective oral intestinal vaccine in the rabbit diarrhea model. The AF/R1 was incorporated into poly D,L-lactide-co-glycolide) **microspheres** and administered intraduodenally. Jarboe et al (34) reported that Peyer's patch cells obtained from rabbits immunized intra-duodenally with AF/R1 in **microspheres** responded with lymphocyte proliferation upon in vitro challenge with AF/R1. This early response at 14 days gave a clear indication as to the immunogenicity of E. coli pili contained within the polymer **microspheres**.

DRWD The CFA/II vaccine has now been incorporated into Poly(D,L lactide-co-glycolide) **microspheres** under Good Manufacturing Practices and tested under Good Laboratory Practices. The **microspheres**, are spherical, smooth surfaced and without pores. The majority (63%) are between 5-10 um in diameter by volume. This. . . was the goal of the vaccine formulation. One percent was chosen because 0.62% was the core loading of the AF/R1 **microspheres** which were effective. Also a small percentage perhaps 1-5% (35) is anticipated to be taken up from the intestine, a. . . .

DRWD . . . This is compared to the occupational maximum allowable exposure of 1800 mgm/15 min. Therefore, the heptane contained with the CFA/II **microsphere** vaccine appears to be a safe level. The acetonitrile is very low -0.1 mgm per vaccine dose. The human oral TDLO is 570 mgm/Kg (any non lethal toxicity). Therefore, the acetonitrile contained with the CFA/II **microsphere** vaccine appears to be at a safe level. The CFA/II vaccine was produced under sterile conditions. However, the process of incorporation of the desalted CFA/II vaccine into the polymer **microsphere** batches and subsequent pooling and loading final dose vials was done in a clean room as for any oral medication. . . .

Ty 21 a oral). Two hundred non pathogenic bacteria are allowed as well as 20 fungi per dose. The CFA/II **microsphere** vaccine is well under these requirements having only 22 non-pathogenic bacteria and 3 fungi per dose.

- DRWD . . . general safety test was also patterned after the WHO requiremets for the TY, 21a oral vaccine in that the CFA/II **microsphere** vaccine was give by gastric lavage to the guinea pigs. Both mice and both guinea pigs demonstrated no toxicity & . . .
- DRWD The CFA/II **microsphere** vaccine (Lot74F2) is immunogenic giving high titer serum IgG antibody responses as early as 7 days following intra muscular injection in rabbits. This test will be used as potency test for future lots of the CFA/II **microsphere** vaccine. Slightly higher antibody titers were seen towards the CS3 pilus protein and this may reflect that CS3 accounts for. . .
- DRWD The CFA/II **microsphere** vaccine was also immunogenic following intra-duodenal administration to rabbits. The highest lymphocyte proliferative responses from Peyer's patch cells were seen. . . the lower 25 ug dose. This is the human equivalent dose and suggests that higher doses of antigen in polymer **microspheres** may attenuate, this immunological reponse.
- DRWD Further evidence of immunization by the CFA/II **microsphere** vaccine given intra-duodenually is demonstrated by the lymphatic hyperplasia in the spleen seen to a greater extend in the rabbits. . .
- DRWD 29. Eldridge, J. H. Gilley, R. M. Staas, J. K. Moldoveanu, Z., Meulbroek, J. A. and Tice, T. r. Biodegradable **microspheres**: vaccine delivery system for oral immunization. Curr. Top. Microbiol, Immunol. 1989, 146, 59-66.
- DRWD . . . K., Gilley, R. M., and Tice, T. R. Controlled vaccine release in the gut-associated lymphoid tissue. I. Orally administered biodegradable **microsphere** target the Peyer's patches. J. Controlled release 2989, 11, 205.
- DRWD . . . Eldridge, J. H. Staas, J. K., Meubroek J. A., McGhee, J. R., Tice, T. R. and Gilley, R. M. Biodegradable **microsphere** as a vaccine delivery system. Mol. Immunol, 1991, 28, 287-294.
- DRWD . . . C. E., Boedeker, E. C., Reid, R. H., Jarboe, D., Wolf, M., Le, M., and Brown, W. R. Pili in **microsphere** protect rabbits for diarrhea induced by E. coli strain RDEC-1. Vaccine (in press).
- CLM What is claimed is:
1. An immunostimulating composition comprising encapsulation-**microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a biodegradable-biocompatible poly (DL-lactide-co-glycolide) or. . .
 4. An immunostimulating composition according to claim 2 wherein the size of more than 50% of said **microspheres** is between 5 to 10 um in diameter by volume.
11. An immunostimulating composition comprising encapsulating-**microspheres**, which may contain a pharmaceutically-acceptable adjuvant, wherein said **microspheres** having a diameter between 1 nanometers (nm) to 10 microns (um) are comprised of (a) a glycolide polymer as a. . .
- AN 95:45359 USPATFULL|
- TI Vaccines against diseases caused by enteropathogenic organisms using antigens encapsulated within biodegradable-biocompatible **microspheres**|
- IN Reid, Robert H., Kensington, MD, United States
Boedeker, Edgar C., Chevy Chase, MD, United States
van Hamont, John E., Shape, Belgium
Setterstrom, Jean A., Takoma Park, MD, United States

09/586747

PA The United States of America as represented by the Secretary of the
Army, Washington, DC, United States (U.S. government)
PI US 5417986 19950523 <--
AI US 1992-867301 19920410 (7)
RLI Continuation-in-part of Ser. No. US 1991-805721, filed on 21 Nov 1991,
now abandoned which is a continuation-in-part of Ser. No. US
1991-690485, filed on 24 Apr 1991, now abandoned which is a
continuation-in-part of Ser. No. US 1990-521945, filed on 11 May 1990,
now abandoned which is a continuation-in-part of Ser. No. US
1990-493597, filed on 15 Mar 1990, now abandoned which is a
continuation-in-part of Ser. No. US 1984-590308, filed on 16 Mar 1984
DT Utility|
FS Granted|
EXNAM Primary Examiner: Henley, III, Raymond J.; Assistant Examiner: Criares,
T. J.|
LREP Lane, Anthony T., Reichert, Earl T., Bellamy, Werten F. W.|
CLMN Number of Claims: 14|
ECL Exemplary Claim: 1|
DRWN 71 Drawing Figure(s); 70 Drawing Page(s)|
LN.CNT 2736|
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L2 ANSWER 20 OF 20 USPATFULL

AB An active agent delivery system for the controlled administration of
macromolecular polypeptides which comprises a micro-suspension of
water-soluble components in a polylactide matrix.
PI US 4962091 19901009 <--
SUMM . . . Contracept. Deliv. Syst. 3: 58; by Sanders et al. (1984),
"Controlled release of a luteinizing hormone-releasing hormone analogue
from poly (d,l-lactide-co-glycolide)-**microspheres**", J.
Pharmaceut. Sci. 73: 1294-1297, by T. Chang, "Biodegradeable
semipermeable microcapsules containing enzymes, hormones, vaccines and
other biologicals", J. Bioengineering, . . .
SUMM Polylactide and poly(lactide-co-glycolide) polymers and copolymers
(referred to generically hereinafter as polylactide or **PLGA**
polymers) are not soluble in water. In contrast, most polypeptides are
soluble in water but not in organic solvents. For. . .
SUMM . . . and extrusion may result in a substantial, often nearly
complete loss of biological activity of the polypeptide. For example, a
PLGA/interferon formulation formed by heated mixing and
extrusion under mild conditions retains less than 1% of the original
biological activity of. . .
DETD . . . and to those which are prepared as copolymers with other
comonomers of the type listed above. The terms poly(lactide-co-
glycolide) and **PLGA** are used interchangeably herein to refer
to copolymers which are prepared as copolymers of lactic and glycolic
acid.
DETD . . . motilin, cholecystokinin, pancreatic polypeptide, gastrin
releasing peptide, corticotropin releasing factor, thyroid stimulating
hormone, vaccine antigens including antigens of HTLV-I, II,
HTLV-III/LAV/**HIV** (AIDS virus), cytomegalovirus, hepatitis A,
B, and non-A/non-B, herpes simplex virus-I, herpes simplex virus II,
malaria, pseudorabies, retroviruses, feline leukemia. . .
DETD . . . resulting supernatant of acetone and water is removed,
additional acetone added, and the mixture vortexed at high speed until
the **PLGA** in the pellet is dissolved, leaving a
micro-suspension of polypeptide and other water-soluble components in
the solution of **PLGA** in acetone.
DETD A. Preparation of IFN/**PLGA** Micro-suspension
DETD One gram of D,L-**PLGA** (molar ratio 50:50, inherent viscosity

0.64 dl/g) was dissolved in 5 ml acetone at room temperature. 0.3 mg of recombinant HuIFN-.beta. in 1 ml of buffer was added to the **PLGA** in acetone and the resulting mixture was vortexed at high speed for approximately 30 seconds. The precipitate of **PLGA**, HSA, IFN and possibly dextrose which formed was then centrifuged for 10 minutes at 700 X g. The supernatant of. . . removed with a cotton swab. Ten ml acetone was added, and the mixture was centrifuged at high speed until the **PLGA** in the pellet was dissolved, leaving a micro-suspension of HuIFN-.beta., HSA and dextrose in a solution of **PLGA** in acetone.

DETD B. Spray-Casting of the IFN/**PLGA** Micro-suspension

DETD The resulting IFN/**PLGA** micro-suspension, obtained as described in paragraph A, was sprayed with an airbrush, using compressed air at 15 PSI, onto a. . . the surface of the sheet and the film sprayed with a constant motion to achieve an even film of the **PLGA** formulation which was approximately 50 microns thick.

DETD Using IFN/**PLGA** micro-suspension from paragraph A, a spray-cast film with silk reinforcement was prepared as follows:

DETD Fine woven silk mesh was stretched on a frame and the stretched portion brushed with a solution of 100 mg/ml **PLGA** (molar ratio 50:50, intrinsic viscosity 0.64) in acetone. The wet mesh was allowed to dry, and then brushed with repeated applications of **PLGA** solution until the pores in the silk mesh were completely filled. The mesh was then dried, placed on a polyethylene sheet, and spray-cast with the IFN/**PLGA** micro-suspension. After drying for one hour, the coated mesh was turned over, coated side down, and again sprayed with the IFN/**PLGA** micro-suspension, applying a layer of polymer about 100 microns thick. After drying for another hour, the previously coated side was. . .

DETD One gram of D,L-**PLGA** (molar ratio 50:50, intrinsic viscosity 0.64 dl/g) was dissolved in 4 ml methylene dichloride. 0.3 mg of recombinant HuIFN-.beta. in. . . 1 ml of buffer containing 12.5 mg/ml human serum albumin (HSA) and 12.5 mg/ml dextrose was added to the dissolved **PLGA** solution. The resulting mixture was vortexed for approximately 60 seconds at high speed until a white emulsion was formed. The. . .

DETD **PLGA**/IFN films prepared as described in Examples 1 and 2, above, were analyzed to determine the particle size of the interferon.

DETD A. **PLGA**/IFN films prepared as described in Example 1

DETD One gram of D,L-**PLGA** (molar ratio 50:50, intrinsic viscosity 0.64 dl/g) was dissolved in 5 ml acetone at room temperature. 0.3 mg of recombinant HuIFN-.beta. in 1 ml of buffer containing 12.5 mg HSA and 12.5 mg dextrose was added to the **PLGA** in acetone and the mixture was vortexed at high speed for approximately 10 seconds. The precipitate of **PLGA**, HSA, IFN and possibly dextrose which formed was then centrifuged for 10 minutes at 700.times. g. The supernatant of acetone. . . removed with a cotton swab. Ten ml acetone were added, and the resulting mixture centrifuged at high speed until the **PLGA** in the pellet was dissolved, leaving an IFN, HSA, dextrose precipitate suspended in **PLGA** dissolved in acetone. A drop of the suspension was viewed under a polarizing light microscope on a glass slide with. . .

DETD The particle sizes of the solid macromolecular components (IFN, HSA, dextrose) suspended in the **PLGA**/acetone solution ranged from less than or equal to the limit of detection (approximately 100 to 500 nanometers) to 100 microns.. . .

DETD B. **PLGA**/IFN films prepared as described in Example 2

DETD A drop of the **PLGA**/IFN micro-suspension prepared according to the method described in Example 2 above was viewed under a polarizing

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light microscope on a. . . .

DETD . . . of this invention, whereby the polypeptide and polylactide are combined and mixed in a heat extrusion apparatus. Ten grams of D,L-**PLGA**, (molar ratio 50/50, intrinsic viscosity 0.64 dl/gm), was mixed with the contents of 25 vials of lyophilized human recombinant interferon. . . .

AN 90:78226 USPATFULL

TI Controlled release of macromolecular polypeptides

IN Eppstein, Deborah A., Palo Alto, CA, United States
Schryver, Brian B., Redwood City, CA, United States

PA Syntex (U.S.A.) Inc., Palo Alto, CA, United States (U.S. corporation)

PI US 4962091 19901009 <--

AI US 1986-866625 19860523 (6)

DT Utility

FS Granted

EXNAM Primary Examiner: Thexton, Matthew A.; Assistant Examiner: Kilby, Catherine S.

LREP Johnson, Lester E., Moran, Tom M., Krubiner, Alan M.

CLMN Number of Claims: 42

ECL Exemplary Claim: 1

DRWN 1 Drawing Figure(s); 1 Drawing Page(s)

LN.CNT 1235

CAS INDEXING IS AVAILABLE FOR THIS PATENT.